

# **NEBRASKA NEWBORN SCREENING PROGRAM**

## **PRACTITIONER'S MANUAL**



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## ACKNOWLEDGMENTS

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Information from the former Practitioner's Manual has been posted on Nebraska's Newborn Screening Web Site [www.dhhs.nnsp/NBS](http://www.dhhs.nnsp/NBS) for the past several years. Numerous changes, most notably the explosion of the number of conditions screened, and new strategies for ensuring successful screening of NICU admissions have taken place over the last decade. This manual is designed to provide the newborn's Health Care Practitioner with the information they need to fulfill their responsibilities. Health Care Provider's most often the newborn's Pediatrician or Family Physician providing pediatric care has primary responsibility for educating the new parent about newborn screening, ensuring the screen is ordered and that any needed follow-up occurs. This manual prepares you for this and provides references to the resources you will need at the time you need them. The State Newborn Screening Program with the experts and stakeholders from the Nebraska Newborn Screening Advisory Committee are here to support you with patient education resources, guidelines and information about regulatory requirements. Nebraska's program is proud of it's recognition for high quality follow-up assistance to Health Care Providers as well as it's mechanisms for continually monitoring and responding to ensure high quality throughout the system.

I wish to acknowledge the substantial contributions in reviewing and editing this manual by the members of the Nebraska Newborn Screening Advisory Committee and the Program Follow-up Coordinator Krystal Baumert and Follow-up Specialist, Karen Eveans. Special recognition goes to Pediatric Subspecialists John Colombo, MD, Kevin Corley, MD, James L. Harper, MD, Richard Lutz, MD, William Rizzo, MD for their expertise on the technical sections covering cystic fibrosis, the endocrine conditions, hemoglobinopathies, and metabolic conditions.

It is our sincere wish that this manual serve as a useful tool to Health Care Providers and invite comment at anytime for the betterment of the system.

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FOR THE MOST UP TO DATE INFORMATION ON NEBRASKA'S NEWBORN SCREENING REQUIREMENTS  
CALL 402 471-6733 OR GO TO

[www.dhhs.ne.gov/publichealth/pages/nsp.aspx](http://www.dhhs.ne.gov/publichealth/pages/nsp.aspx)

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## **INTRODUCTION**

Nebraska was one of the original 30 states to participate in the phenylketonuria (PKU) trials in the 1960's. By 2008 Nebraska was screening for 28 conditions from the blood spot specimen. Proposals to screen for an ever increasing cadre of conditions undergo evidence-based review by the Federal Health and Human Services Secretary's Advisory Committee on Heritable Diseases in Newborns and Children (ACHDNC), as well as Nebraska's Newborn Screening Advisory Committee (NBSAC). The NBSAC also considers factors such as what resources must be in place for a state-wide screening system to successfully add any particular condition.

## **PURPOSE OF THIS MANUAL**

The purpose of this manual is to give clear and concise information to Nebraska practitioners involved with newborn screening. It gives information on screening practices and a general overview of each disorder. It includes a listing of many on-line resources available to newborn health care providers as additional tools and information. The regulations and the State Statute are included in the appendix.

The goal of the Nebraska Newborn Screening Program (NNSP) is to assure the timely and appropriate diagnosis and treatment of Nebraska newborns affected by the disorders screened. This manual is designed to enhance the practitioner's knowledge of the Nebraska Newborn Screening Program to facilitate screening of all infants for the specified conditions.

We encourage practitioners to use the term "newborn screen" or "bloodspot screen" rather than "PKU test" since many other disorders are included in the screening battery, and to avoid confusion with the point-of-care screening done for early hearing detection and intervention.

## **GENERAL OVERVIEW OF THE NEWBORN SCREENING PROGRAM AND THE HEALTH CARE PROVIDER'S ROLE**

Every baby born in Nebraska is required by law to be screened.

Parent education ideally occurs before the baby is born, sometime during the third trimester. Obstetric care providers are encouraged to introduce information about newborn screening during the third trimester. Copies of a one-page introductory parent information sheet can be obtained from the NNSP at no charge. The "Parent's Guide to Your Baby's Newborn Screening" brochure is also provided at all birthing facilities to give to new parents either at pre-admission or during the labor/delivery and post-partum hospital stay. This is available in 10 languages. A video for parents "Newborn Screening, Protecting Your Baby's Health" has also been provided to all hospital mother/baby units, and is available in English and Spanish. Copies of any of these materials are available free of charge to practitioner's for use in their clinics.

After the baby is born in the hospital, newborn screening specimens are ordered. Five drops of blood are collected via heel stick on a standard filter paper form (the CARE form or Collection and Reporting Form). This should occur when the baby is 24 to 48 hours old, or immediately prior to discharge, whichever comes first. The form is allowed to dry at room temperature for about 4 hours and is shipped overnight to the testing laboratory within 24 hours of collection.

Once the testing laboratory receives the specimen, an accession number is assigned and all information is entered into the newborn screening database in the lab, and concurrently laboratory testing begins. In-lab turn around time is generally about 1.5 days to complete all the testing, although some results are available earlier.

If all tests are normal, the lab sends the test results to the hospital. The hospital makes the test results available to the ordering physician.

If the specimen is determined to be unsatisfactory for testing, or if one or more tests are inconclusive or positive, the lab will notify the submitter and the newborn's physician (determined from the CARE form) which of the tests need to be repeated or need other confirmatory/diagnostic testing. The State Newborn Screening Program will also contact the baby's health care provider with further information for referral and diagnostic testing for positive screens and provide parent information sheets and Physician ACT (action) sheets. In urgent situations as determined by established protocols, the program will provide the information to the appropriate pediatric sub-specialist and connect them with the attending physician. Follow-up action by the health care provider should be done without delay to ensure the well being and safety of the child.

On average, when all goes as planned (collection between 24-48 hours, overnight shipping, and in-lab testing and reporting), results are available between 4.5-5 days of age. Rapid turn around time is critical to identify affected newborns and prevent neurological damage, other chronic health problems and death.

The State Newborn Screening Program follow-up personnel monitor and track every baby requiring any additional testing. They will stay connected with you via phone, fax and mail until necessary repeat or confirmatory testing has been completed and the program receives documentation either ruling out a condition, or verifying diagnosis and that treatment has begun. In most cases only a repeat screen is required. For most repeats we can track this via the laboratory data system. If repeat testing is conducted by another State newborn screening lab, or whenever any other confirmatory testing is required, the attending physician must report the results back to the newborn screening program including diagnosis and treatment information as appropriate.

### Common Questions & Answers

**Q:** I have a parent who wishes to refuse the newborn blood-spot screening. Is there a waiver form they can sign?

**A:** No. Neb.Rev.Stat §§ 71-519 to 71-524 is explicit that "all newborns" born in Nebraska must be screened. The law holds the baby's attending physician responsible for ensuring the newborn screen is completed and that any needed follow-up occurs. In addition to the routine education you provide to parents about the benefits of newborn screening and the minimal risk of the heel stick procedure, it may be helpful to

explain that the newborn screening law has been challenged several times in County, District, and Juvenile Court. Each time the ruling has been the baby must be screened. An appeal to the Nebraska Supreme Court also upheld the law that every newborn must be screened.

Q: I ordered the baby's newborn screen but I am not following this baby after discharge. Whose responsibility is it for follow-up?

A: The practice of having a Hospitalist or on-call physician or other health care provider see the baby in the hospital and order the newborn screen is becoming more common. The ordering physician is listed on the "Collection and Reporting Form" attached to the filter paper which functions as the laboratory test requisition. Results must be reported back to the ordering physician. You are responsible for ensuring appropriate follow-up. If you know you will not be seeing the baby post-discharge you should ensure that upon discharge an attending physician or other health care provider is identified by the parents and recorded in the baby's medical record. When you are notified of an abnormal result after discharge and you are no longer seeing the baby, you should contact the identified baby's physician to ensure they know of the abnormal result, and the repeat or confirmatory testing that must be done. If the identified post-discharge physician denies knowledge of the patient, you should follow-up directly with the parents to ensure appropriate follow-up occurs. When personnel from the laboratory or state follow-up program contact you with abnormal screen results, you should notify them if you are not the baby's physician, and who is identified as the baby's post-discharge physician.

Q: I'm hearing concerns from parents about the Government keeping their baby's DNA? How can I respond with good information.

A: Different States have different practices on how long they keep any leftover dried blood spot specimens, as well as how they may be used. In Nebraska we do not keep the blood spots long term. They are kept for 90 days, and then in the following 30 day period they must be destroyed. Our laboratory ensures they are incinerated. If during the short period that they are stored, someone wishes to use them for research, they can only do this if they first have IRB approval, parent/guardian consent and the approval of the Department of Health and Human Services' Chief Medical Officer. Only then can they be released, and only after the first 90-day storage period, because during that time the priority use of the specimen must be for the benefit of the baby. For example, some babies who are identified with hearing loss, benefit from further testing of the residual dried blood spot to identify if they had congenital cytomegalovirus (vs. acquired CMV) which may explain the hearing loss, and perhaps rule out other syndromic causes of hearing loss.

If you are caring for a baby born and screened in another State and parent's have questions about residual blood spot storage, you can advise the parent's to check with that State's Newborn Screening Program about their policies for storage, use and disposal of residual dried blood spots. Contacts for all State Program's can be found at the National Newborn Screening and Genetics Resource Center's web-site [www.genes-r-us.uthscsa.edu](http://www.genes-r-us.uthscsa.edu)

Q: Just how important is it to get the specimen collected at 24-48 hours of age?

A: Every attempt is made to control variables that can affect test results in population screening in order to improve the sensitivity and specificity of results. In the case of timing, almost all reference ranges are

based on specimens collected between 24-48 hours of age. For some conditions such as congenital primary hypothyroidism, the newborn has substantial changes in thyroxin (T4) and TSH during the first 24 hours, with recognizable patterns of normal and abnormal results in the next 24 hour period. For aminoacidopathies such as PKU where phenylalanine and phenylalanine to tyrosine ratios are important indicators, it is important to get past the initial catabolic phase, although due to the sensitivity of tandem mass spectrometry testing, it is no longer important that the baby have 24 hours of feedings in order to detect elevations of phenylalanine suspicious of PKU. Many of the conditions screened can affect the baby within days or weeks, so rapid intervention is essential, and collecting the specimen at the right time can help this.

Q: What if a specimen still hasn't been collected by 48 hours of age?

A: It is still essential to collect a newborn screen. Although the longer one waits, the higher the risk of delayed diagnosis and treatment for affected newborns and hence the increased risk for irreversible neurological damage, other health problems and infant death.

Q: I have a patient who delivered their baby at home and has not been to the hospital. What do I need to do?

A: All out of hospital births must also be screened. Order the newborn screen immediately and help the parent's navigate where to go. All birthing facilities have the ability to collect the specimens.

Q: What are the key things that can help me explain the importance of newborn screening to parents of my patients?

A: Number one, babies with these conditions usually don't have signs and symptoms that are recognizable until the internal damage has begun. So the blood test gives us a head start to be able to find it.

We screen for these conditions because there are treatments available, and if started early enough we can prevent serious problems like neurological damage and infant death, and at a minimum in other cases we can reduce the effects of the condition.

The parents might not have any family history (or known family history) for any of these conditions. In fact, in most cases when we find a new baby with one of these conditions, they do not have any known family history.

Q: I had a patient worried about their child's insurability if they have one of the screened conditions. What should I tell them?

A: Insurability may be an issue whether the child's condition is detected early or detected too late. If the child is detected early, treated, and damage is prevented, or if the child isn't screened, presents with signs and symptoms (such as a metabolic crisis) and suffers severe neurological damage requiring a lifetime of care the insurance issue will probably be the same. The Federal Genetic Information Non-Discrimination Act (GINA) passed in 2008 has some protective provisions relative to denying coverage for pre-existing



conditions.

Q: Some parents react strongly with anxiety when we have them bring baby back for repeat or confirmatory testing. How can I balance the need for them to respond rapidly, with reassurance to minimize their anxiety?

A: Helping parents to understand the concept that “screening” is like casting a large net to catch a few fish might be useful. Finding those few babies that are affected but are difficult to find because they look so healthy at birth is the goal. So, sometimes there will be “false positives” or results that when they get retested, come back normal the second time. On the other hand, if the screen result was correct, and is verified by repeat or confirmatory/diagnostic testing, this is the best chance to help their baby live a healthy life, by finding out early and getting treatment. The more you understand about the special considerations for each test (see “Special Considerations” following the description of each screened condition), the better prepared you will be to answer further questions, and help explain other reasons why “false positives” sometimes occur.

Q: Why do I hear that some states are screening for as many as 55 conditions and we only screen for 28?

A: With a few exceptions, generally all states are screening for the same conditions or at least looking at the same analytes. The vast difference is in the way states identify the conditions that can be detected via tandem mass spectrometry. This test looks at multiple markers for acylcarnitines and amino acids. The core panel of 28 ACHDNC recommended conditions, includes 20 diseases from this one test. A secondary target group includes another large group from this same test. They are considered secondary targets for a few reasons. Primarily they are typically even more rare than those in the core group, but they might be identified through differential diagnosis when a screen result comes up abnormal. Another reason some are on a “secondary target” list is that there is less evidence to support that they can be reliably detected in the newborn screen. Some may not have detectable elevations or abnormalities at the time most screening specimens are collected. And finally some of the conditions on the secondary target list may be so rare that the best course of treatment or the efficacy of treatment may not be known.

Q: Can I get results STAT if I have a concern about a baby?

A: It is perfectly acceptable, and preferable to contact the newborn screening program (402) 471-0374 or laboratory (412) 220-2300 if you have a sick baby and suspect a possible screened condition. The laboratory will prioritize those requests for processing and contact you with the results.



## RECOMMENDED ON-LINE RESOURCES FOR PROFESSIONAL MEDICAL PERSONNEL

Nebraska Newborn Screening Program Web-site: [www.dhhs.ne.nsp](http://www.dhhs.ne.nsp)

Includes a Program Overview, Sections for Health Care Practitioners, Hospitals and Parents, Regulations and Statute, Publications, and Links to useful resources

National Newborn Screening and Genetics Resource Center Web-site: [www.genes-r-us.uthscsa](http://www.genes-r-us.uthscsa)

National Data on State Reported statistics, program descriptions, Links to useful resources

American College of Medical Genetics ACT Sheets: [www.acmg.org](http://www.acmg.org)

Condition specific Action sheets to help physicians respond to positive newborn screen result

National Library of Medicine Genetic Home Resource pages: <http://ghr.nlm.nih.gov>

Includes specific descriptions of screened disorders, genotype, phenotype.

## NEBRASKA SPEAKERS, EXPERT RESOURCES AVAILABLE TO YOU

Contact the Nebraska Newborn Screening Program for contact information.

TOPIC	PERSON	AFFILIATION
Cystic Fibrosis	John Colombo, MD	Director Cystic Fibrosis Center, member of NBS Advisory Committee
Endocrine conditions screened	Kevin Corley, MD	Pediatric Endocrinologist, UNMC/Children's, member NBS Advisory Committee
Endocrine conditions screened	Richard Lutz, MD	Geneticist, Pediatric Endocrinologist, UNMC/Children's member of NBS Advisory Committee
Hemoglobinopathies screened SCID	James L. Harper, MD	Pediatric Hematologist, UNMC/Childrens, member of NBS Advisory Committee
Newborn screening Metabolic conditions screened Endocrine conditions screened Use of MS/MS in NBS	Richard Lutz, MD	Board certified Pediatrics, Medical Genetics, Pediatric Endocrinology
Metabolic conditions screened	William Rizzo, MD	Board certified Pediatrics, Biochemical Genetics
NICU practices and newborn screening	Khalid Awad, MD	Board certified Pediatrics, Neonatology Perinatology, Medical Director, Methodist Women's Hospital NICU, Member NBS Advisory Committee
Newborn Screening Practices, Regulation requirements	Julie Luedtke	Program Manager, Nebraska Newborn Screening Program 402 471-6733

## DISORDERS ON NEBRASKA'S NBS PANEL

The following table (1) shows conditions in Nebraska's screening panel and which tests are used. Estimated incidence rates are derived from the National Newborn Screening and Genetics Resource Center data reported by states of confirmed cases from 2008 (the most recent year available at the time of publication). Those rates assume 4,255,198 births screened. In some cases State screening data reported did not reflect the full year as the particular State did not begin screening for a particular condition until sometime in the year.

For perspective, using the current panel of 28 conditions screened, Nebraska screens approximately 26,000-27,000 babies per year, and identifies 35-50 newborns with clinically significant conditions each year.

**TABLE 1**

<b>Information Related to Disorders Included on Nebraska's Screening Panel</b>				
<b>Condition / Year screening started in Nebr.</b>	<b>Test Method and Analyte</b>	<b>Estimated Incidence (U.S.)*</b>	<b>Symptoms If Not Treated</b>  (age at onset, spectrum of symptoms and severity of presentation may be variable/ patient specific)	<b>Treatment</b>
Argininosuccinic Acidemia (ASA Lyase) 2003 supplemental 2008 required 📌	Tandem Mass Spectrometry (MS/MS) Amino Acid (AA) profile Arginine	.003/1000	Poor feeding, lethargy, hypotonia, tachypnea, seizures and signs of liver disease. hyperammonemia. mental retardation, seizures and spastic diplegia.	Low protein, L-amino acids, L-carnitine, sodium benzoate, L- arginine,
Beta Ketothiolase Deficiency (BKT) 2003 supplemental 2008 required	MS/MS Acylcarnitine (AC) profile C5, C5:1 with C5:OH	.000/1000 (in 2008 only 2 cases reported in U.S. NBS data)	Poor feeding, vomiting, lethargy, hypoglycemia, hypotonia, ketonuria, metabolic acidosis, mild developmental delay.	Protein restriction, avoid ketogenic diet, carnitine supplementation
Biotinidase Deficiency (BIO) 1987	Enzyme assay Biotinidase	.013/1000 profound  .031/1000 partials  1:72,000 to 1:126,000	Mental retardation, seizures deafness, blindness, desquamating eczema	Pharmaceutical doses of Biotin
Carnitine Uptake Defect (CUD) 2003 supplemental 2009 required	MS/MS Acylcarnitine profile (AC) CO Carnitine	.007/1000	Tachycardia, hepatomegaly, reduced muscle tone, lethargy, hypotonia, hepatomegaly, cardiac decompensation due to cardiomyopathy.	Supplemental carnitine.

Information Related to Disorders Included on Nebraska's Screening Panel				
			Hypoglycemia in acute episodes.	
Citrullinemia (CIT) 2003 supplemental 2008 required	MS/MS AA profile Citrulline	.005/1000	Poor feeding, vomiting, lethargy, hypotonia, tachypnea, seizures, hyperammonemia, and signs of liver disease.	Protein restricted diet, may include L-amino acids, L-carnitine, L-arginine & some phenylbutyrate supplementation
Congenital Adrenal Hyperplasia (CAH) 2006	Steroid test: 17-hydroxyprogesterone (17-OHP)	.044/1000	Ambiguous genitalia, lethargy, vomiting, poor feeding, risk of life-threatening adrenal crisis, shock and death.	Emergency IV fluids, glucose, IM/IV hydrocortisone for adrenal crisis. Hormone therapy.
Congenital Primary Hypothyroidism(CPH) 1979	Thyroid hormones: T <sub>4</sub> and TSH	.463/1000	Developmental delay, mental retardation and poor growth	Thyroid hormone replacement
Cystic Fibrosis 2006	Immuno-assay Immuno-reactive trypsinogen (IRT)	.128/1000	Abnormally thick mucus leading to airway obstruction, neutrophil dominated inflammation and recurrent and progressive pulmonary infections. Pancreatic insufficiency in 80-90% of cases can result in malnutrition, poor growth.	Enzymes, vitamin supplementation, close monitoring, prevent and treat infections, airway clearance.
Galactosemia 1996	Total Galactose and enzyme; Galactose-1-Phosphate Uridyl-Transferase (GALT)	.027/1000 1:60,000 to 1:80,000	Septicemia, cataracts, mental retardation, cirrhosis, ovarian failure, death if untreated	Galactose-free diet
Glutaric Acidemia Type I (GA-I) 2003 supplemental 2008 required	MS/MS ACprofile C5-DC or C5:OH with C8 & C16	.010/1000	Macrocephaly, muscle hypotonia, metabolic ketoacidosis, failure to thrive, sudden onset dystonia and athetosis	Protein restriction, riboflavin, carnitine supplementation, and possibly lysine/tryptophan restricted formula
Hemoglobinopathies -Sickle Cell Disease - Hgb. C Disease - Thalassemias 1996	Isoelectric focusing: Hemoglobin patterns for hemoglobins F, A, S, C, variants & Barts	.131/1000 (combined SS, SC, SA)  1:40,000 to 1:60,000 * 1:375 SS ** 1:835 SC † 1:1,667 ‡	Anemia, septicemia, dactylitis, acute chest syndrome, splenic sequestration, pain crises, strokes, high mortality rate	penicillin prophylaxis, folate supplementation, other treatments available depending on type/course of disease
Homocystinuria (HCY) 2003 supplemental	MS/MS AA profile for methionine	.001/1000	Mental retardation, ectopia lentis, marfanoid appearance including arachnodactyly,	Low protein diet, L-amino acids, L-carnitine, Cystadane,

Information Related to Disorders Included on Nebraska's Screening Panel				
2008 required			osteoporosis and other skeletal deformities, and thromboembolism.	folate
3-Hydroxy-3-Methylglutaric Aciduria (HMG) 2003 supplemental* 2008 required	MS/MS AC profile for C5:OH, C6-DC (with C5:OH)	.006/1000	Hypoglycemia, ketonuria, metabolic acidosis, poor feeding, vomiting, lethargy, developmental delay	Avoid fasting to prevent hypoglycemia , avoid excess fat to prevent ketoacidosis
Isovaleric Acidemia (IVA) 2003 supplemental 2008 required	MS/MS AC profile: C5, C6-DC with C5:OH	.006/1000	Metabolic ketoacidosis, dehydration, hyperammonemia, ketonuria, hypoglycemia, poor feeding, vomiting, lethargy, tachypnea, sweaty feet odor, failure to thrive.	Low protein, low leucine, L-carnitine & glycine supplementation
Long-Chain Hydroxy Acyl-CoA Dehydrogenase Deficiency (LCHAD) 2003 supplemental 2008 required	MS/MS AC profile: C16:OH, and C16 with C18:1	.003/1000	Hepatomegaly, cardiac myopathy, lethargy, hypoketotic hypoglycemia, elevated liver transaminases, elevated creatine phosphokinase (CPK), lactic acidosis, rhabdomyolysis, failure to thrive	Avoid fasting, carnitine supplementation. Alter dietary fat intake.
Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD) 2002	MS/MS AC profile: C8, C8:C16 ratio	.051/1000	Critical hypoketotic hypoglycemia, lethargy, vomiting and sudden death.	Avoid fasting, L-carnitine supplement, low fat, low medium chain fatty acid diet
Methylmalonic Acidemia (Mutase) (MMA) 2003 supplemental 2008 required	MS/MS AC profile: C3, C3:C2 and C3:C16 ratios	.008/1000	Metabolic ketoacidosis, dehydration, hyperammonemia, ketonuria, hypoglycemia poor feeding, vomiting, lethargy, tachypnea, failure to thrive. Long term complications common.	Low protein, L-amino acids, L-carnitine, Vitamin B-12, hospitalize for acute episodes
Methylmalonic Acidemia (Cbl A,B & C) (MMA) 2003 supplemental 2008 required	MS/MS AC profile: C3, C3:C2 and C3:C16 ratios	.002/1000	Metabolic ketoacidosis, dehydration, hyperammonemia, ketonuria, hypoglycemia poor feeding, vomiting, lethargy, tachypnea, failure to thrive. Long term complications common.	Protein restriction, consider dietary formula, Betaine, B <sub>6</sub> , folate and carnitine. IM Hydroxycobalamin.
3-Methylcrotonyl-CoA Carboxylase Deficiency (3-MCC) 2003 supplemental 2008 required	MS/MS AC profile: C5:OH, or C5:1 with C5:OH	.015/1000	Hypoglycemia, ketonuria, metabolic acidosis, poor feeding, vomiting, lethargy, developmental delay	Dietary based low protein diet to limit leucine. Carnitine supplement. May be responsive to biotin.
Multiple Carboxylase Deficiency (MCD) 2003 supplemental 2008 required	MS/MS AC profile C3 or C5-OH	.000/1000 (2 cases in 2008 in U.S.)	Hypoglycemia, ketonuria, metabolic acidosis, poor feeding, vomiting, lethargy, developmental delay	High dose oral biotin. Treat/hosp acute episodes
Maple Syrup Urine Disease (MSUD) 2003 supplemental	MS/MS AA profile Valine, Leucine,	.004/1000	Poor feeding, vomiting, lethargy, tachypnea, alternating hypertonia/hypotonia, seizures,	Low leucine, Isoleucine, valine. L-carnitine, thiamine,

Information Related to Disorders Included on Nebraska's Screening Panel				
2008 required	Isoleucine		metal retardation, hyperactivity, failure to thrive, coma, cerebral edema and possibly death	glycine supplement
Phenylketonuria (PKU) 1967	MS/MS AA Profile phenylalanine and tyrosine	.039/1000 1:10,000 to 1:25,000	Mental retardation, hyperactivity, autism, seizures, eczema, odor	Low phenylalanine metabolic formula and foods
Propionic Acidemia (PA) 2003 supplemental 2008 required	MS/MS AC profile C3, C3:C2, C3:C16	.004/1000	Metabolic ketoacidosis, dehydration, hyperammonemia, ketonuria, hypoglycemia poor feeding, vomiting, lethargy, tachypnea, failure to thrive. Long term complications common.	Low protein, L-amino acids, L-carnitine, biotin. Treat/hosp for acute episodes
Tri Functional Protein Deficiency (TFP) 2003 supplemental 2008 required	MS/MS AC profile C16-OH or CF18-1-OH with C16:OH or C16:OH with C18:10H, C16, C18:1 or C14:1	.000/1000 (1 case in U.S. in 2008)	Hypoglycemia, cardiomyopathy, neuromyopathy, neonatal liver failure or heart failure.	Avoid fasting, carnitine supplementation. Alter dietary fat intake.
Tyrosinemia (TYR) Types 1, 2, 3, 2003 supplemental 2008 required	Tyrosine	.001/100 type 1 .001/1000 type 2 no data type 3	Tyr I – liver disease and cirrhosis. Tyr II hyperkeratosis of skin, corneal ulcers and some mental retardation. Tyr III may be benign	Varies by type, restricted diet, may use formula supplements, Nitisinone
Very Long Chain Acyl-CoA Dehydrogenase Deficiency (VLCAD) 2003 supplemental 2008 required	MS/MS AC Profile C14, C14:1, C14:2 and C14:1 to C12:1 ratio	.016/1000	Poor feeding, lethargy, hypotonia, hepatomegaly, arrhythmia, evidence of cardiac decompensation, hypoketotic hypoglycemia and failure to thrive.	Avoid fasting, carnitine supplementation. Alter dietary fat intake. MCT oil for some.

\* Incidence of SS (sickle cell disease) in non-African-American newborns in California, New York and Texas

\*\* SS = sickle cell anemia in U.S. African-American live births

† SC = SC disease in U.S. African-American live births

‡ HbS β-thalassemia in U.S. African-American live births

☞ In 2003 when tandem mass spectrometry screening for MCAD became mandatory, the rest of the MS/MS panel results were initially offered to parents. Approximately 97% of parents opted to receive those results. In 2008 MS/MS results for all 20 core conditions recommended by the ACHDNC for the “Recommended Universal Screening Panel” (RUSP) became mandatory via regulation in Nebraska.

TABLE II

SUMMARY OF TEST RESULT INTERPRETATIONS AND RECOMMENDED PROTOCOL FOR RESPONDING TO ABNORMAL RESULTS		
Condition Screened and cut-offs	Interpretation of result (WNL = within normal limits)	Practitioner's response
<b>Biotinidase Deficiency</b> $>8.1-16$ ERU  $\leq 8$ ERU	Inconclusive for biotinidase deficiency	Specimen has low enzyme activity but not in the typical profound deficiency range. Possible heat denatured specimen, profound, partial, carrier or false positive. Obtain a repeat dried blood spot specimen.
	Positive for biotinidase deficiency	Specimen has substantially low enzyme activity on initial screen, or continued low enzyme activity on second screen. Order a confirmatory serum specimen (with unrelated control specimen) per NBS program instructions. Consult with pediatric metabolic specialist.
<b>Congenital Adrenal Hyperplasia</b>  Every specimen collected at $> 24$ hours is tested for 17-OHP and reference ranges are adjusted based on birthweight.  Some reflex to an extracted-17OHP to eliminate some of the noise from interfering substances often associated with prematurity.  There are several classifications of abnormal results reported out, with specific protocols recommended for each. These are designed to minimize anxiety and cost associated with high false positives that can be common with the CAH assay.	Specimen has an elevated 17-OHP above the critical cut-off and the extracted 17-OHP is pending and birthweight is $> 2500$ g. <b>POSITIVE</b> ----- Specimen has an elevated 17-OHP above the critical cutoff, but the infant is $< 2500$ grams. Extracted 17-OHP is pending <b>INCONCLUSIVE</b> ----- Specimen has an elevated 17-OHP but not above the critical cut-off, extracted 17-OHP is elevated, and baby's birthweight is $<1500$ g.. <b>INCONCLUSIVE</b> ----- Specimen has an elevated 17-OHP but not above the critical cut-off and extracted 17-OHP is elevated, and the baby's birthweight is $>1500$ g. <b>INCONCLUSIVE</b>	<b>Possible neonatal emergency.</b> Immediately assess the infant for signs of salt-wasting., admit to hospital for observation, consult with pediatric endocrinologist, and monitor electrolytes. Order Steroid Profile designated by specialist. Extracted 17-OHP from dried blood spot will be reported as soon as available.  ----- Request parent bring baby to clinic visit for assessment the day of notification. Consult with pediatric endocrinologist. Recommend Steroid Profile testing. If VLBW 17-OHP1 acceptable as requires less blood. Extracted 17-OHP will be reported out as soon as available. [If elevated, amended report of <b>POSITIVE</b> will be made, if normal amended report of <b>WNL</b> will be made.]  ----- Recommend repeat dried blood spot testing.  ----- Recommend repeat dried blood spot testing.

SUMMARY OF TEST RESULT INTERPRETATIONS AND RECOMMENDED PROTOCOL FOR RESPONDING TO ABNORMAL RESULTS		
Condition Screened and analytes tested	Interpretation of result (WNL = within normal limits)	Practitioner's response
<b>Congenital Primary Hypothyroidism</b> All newborn specimens collected at > 24 hours are tested for T4. The lowest 10% of T4's reflex to TSH. If TSH $\geq$ 20 ng/mL is reported out abnormal	T4 lowest 10% of run  TSH $\geq$ 20 mIU/L  Positive screen result for congenital primary hypothyroidism	Obtain serum FT4 and TSH. Consult with pediatric endocrinology. May suggest nuclear thyroid studies.
<b>Cystic Fibrosis</b> All specimens tested for immunoreactive-trypsinogen (IRT). Elevated IRT's > 90 reflex to look for most common mutation associated with CF, $\Delta$ F508. If present, reflexes again to larger mutation panel.  Note: If baby presents with Meconium Ileus or other bowel obstruction: report to screening lab. Test will automatically reflex to 39 + 4 CFTR mutation panel.	IRT > 90 and no $\Delta$ F508  IRT > 90 and one copy $\Delta$ F508  IRT > 90 and two copies of $\Delta$ F508, or one copy $\Delta$ F508 with another mutation from the 39 + 4 CFTR mutation panel  MI: IRT results are often low, even though these babies are at increased risk of having cystic fibrosis. The IRT is not considered a reliable screen for babies with MI or other bowel obstruction.	Inconclusive screen for CF. Obtain repeat DBS.  Inconclusive screen for CF. At least a carrier. Refer to Accredited CF Center for sweat testing and evaluation.  <b>POSITIVE</b> for Cystic Fibrosis. Refer to Accredited CF Center for sweat chloride testing and evaluation.  Report MI to laboratory. Testing will proceed on the mutation panel. Consider consult or referral to CF Specialist for evaluation.
<b>Galactosemia</b> The screen for galactosemia tests for galactose and GALT (uridyl transferase). Gal > 15 positive. Results assessed in conjunction with GALT.	Elevated galactose with normal GALT  Inconclusive screen result for Galactosemia, possible Duarte homozygote, Galactokinase Deficiency, or false positive ----- Elevated galactose with	Obtain repeat dried blood spot specimen.  ----- <b>POTENTIAL NEONATAL EMERGENCY- Consult pediatric metabolic specialist. Interrupt breast or cows milk formula</b>



SUMMARY OF TEST RESULT INTERPRETATIONS AND RECOMMENDED PROTOCOL FOR RESPONDING TO ABNORMAL RESULTS		
	<p>low GALT enzyme activity.</p> <p>Positive screen result for Galactosemia</p>	<p>feedings. Collect confirmatory whole blood and order galactose-1-phosphate uridylyltransferase assay. Assess infant. May wish to check urine for reducing sugars. Consult pediatric metabolic specialist. Introduce powder-based soy formula after confirmatory specimen collected.</p>
<p><b>Hemoglobinopathies</b></p> <p>Results reported out in order of highest quantity to lowest.</p> <p>F= fetal hgb.</p> <p>A= Adult hgb.</p> <p>S= Sickle hgb.</p> <p>C= Hgb. C</p> <p>Barts= Barts hgb.</p> <p>V= all other variants</p>	<p>Anything other than FA is not a normal hgb. Screen result.</p>	<p>Collect and order a newborn hemoglobinopathy electrophoresis confirmatory specimen.</p> <p>Most of the findings will be indicative of trait or carrier status.</p> <p>However, screen results of:</p> <p>FS, FSC, FC, FS+ Barts, FSA, F only, and FE are all likely clinically significant. FA + Bart's could involve a clinically significant thalassemia and should be followed to determine if one, two or three gene deletion.</p>
<p><b><u>Amino acidopathies:</u></b></p> <p>-ASA</p> <p>-CIT</p> <p>-HCY</p> <p>-MSUD</p> <p>-PKU</p> <p>-TYR</p> <p><b><u>Fatty acidopathies:</u></b></p> <p>-CUD</p> <p>-MCAD</p> <p>-LCHAD</p> <p>-TFP</p> <p>-VLCAD</p> <p><b><u>Organic acidopathies:</u></b></p> <p>BKT</p> <p>GAI</p> <p>IVA</p> <p>MMA(mutase)</p> <p>MMA(Cbl A&amp;B)</p> <p>MCD</p> <p>PA</p>	<p>All are screened by MS/MS. The acylcarnitine profile and amino acid profiles look for markers and relative ratio's of markers to determine results that are out of range.</p> <p>Results are reported out at three levels:</p> <p>1) Slight elevation</p> <p>2) Above normal or moderate elevation</p> <p>3) Substantially elevated</p>	<p>1) When result is reported out as slightly elevated, obtain a repeat dried blood spot newborn screening specimen. Newborn screening regulations require repeat specimens to be collected within 48 hours.</p> <p>2) When result is reported that levels are abnormal, this is a more concerning result and the lab report will recommend obtaining a repeat dried blood spot newborn screening specimen within 48 hours. The NBS follow-up program recommends these be collected sooner.</p> <p>3) When result is reported out as substantially elevated, this is a high alert situation. The newborn screening follow-up program will put you in touch with the pediatric metabolic specialist on service at that time 24/7. You will also receive a copy of a link to an ACT (action) sheet with recommendations for immediate next steps.</p>

SUMMARY OF TEST RESULT INTERPRETATIONS AND RECOMMENDED PROTOCOL FOR RESPONDING TO ABNORMAL RESULTS		
3-MCC HMG		Various confirmatory tests are recommended depending on the suspected condition, quantitative plasma amino acids, urine organic acids, etc. The metabolic specialist will be able to assist with recommending confirmatory tests, as well provide guidance on any needed immediate assessment of the infant . It is recommended these patients be referred to the pediatric metabolic clinic, for diagnosis and management.
	4) Repeat specimen remains above normal	-Refer to laboratory report for recommended response.

† This is a condensed table. Please refer to the regulations (see appendix) for complete duties and responsibilities.

***PLEASE NOTE: THESE TESTS ARE SCREENING TESTS. ABNORMAL RESULTS NEED FULL EVALUATION AND CONSULTATION BEFORE A DIAGNOSIS IS CONFIRMED OR TREATMENT IS STARTED.***

***Treatment or intervention before confirmatory or diagnostic test results are available is ONLY recommended when positive screen results are found for MCAD (counseling on avoiding fasting), Galactosemia (soy formula) and Congenital Adrenal Hyperplasia (hospitalize for saltwasting crises). The pediatric endocrinologist will determine if treatment for congenital primary hypothyroidism should begin before the results of diagnostic tests are received.***

## BIOTINIDASE DEFICIENCY

Biotinidase is an enzyme that releases biotin, an essential vitamin cofactor, from a bound form so that it can be used by the body. Deficiency of the enzyme results in improper functioning of several other enzyme systems dependent on biotin, leading to neurological damage. This recessively inherited disorder affects the regeneration of biotin and leads to greatly reduced carboxylase activity. Nebraska's incidence of Biotinidase Deficiency has been strikingly higher than worldwide. Internationally the incidence of profound biotinidase deficiency is reported at 1:112,000 and partial deficiency at 1:129,000<sup>1</sup>. Nebraska's incidence of 52/474,833 births screened between 1991-2009 was 1:9,131 for partial and profound deficiency combined.

### Clinical Features

The symptoms of biotinidase deficiency are variable with respect to age of onset, frequency and severity. Infants with biotinidase deficiency appear normal at birth but develop one or more of the following symptoms after the first few weeks or months of life: ataxia, seizures, hearing loss, alopecia, developmental delay, skin rash, or metabolic acidosis which can result in coma and death. Individuals with partial deficiency (a variant form) may also be at risk for development of symptoms. Family studies are indicated when an affected newborn is identified.

### Laboratory Tests

Detection of enzyme activity is made by a qualitative colorimetric assay. In the presence of the enzyme, a color change occurs. Via chromatography a semi-quantitative measure converts to a value referenced in units of Enzyme Reactive Units.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
< 8.0 ERU (Positive)	-Biotinidase deficiency -Partial Biotinidase deficiency -Enzyme in specimen denatured by heat and/or humidity -False positive	-POSTIVE: Notify parents, and obtain confirmatory serum specimen per program protocol (specifics will be communicated via phone.) Consult with pediatric metabolic specialist. Report lab tests, diagnosis and treatment to NNSP.
8.1-<16 ERU (Inconclusive)		-INCONCLUSIVE: Notify parents, and obtain repeat dried blood spot testing. Most often levels will normalize on repeat. If on repeat levels are still low, consult with and/or refer to pediatric metabolic specialist.

### Confirmation and Treatment

Consultation with a pediatric metabolic specialist should be made for confirmation and diagnosis (see Medical Consultants). Upon notification of a positive screening result the physician should collect or cause to be collected 0.5ml (minimum) of serum. The specimen must be frozen (sent on dry ice) and should be tested for biotinidase enzyme activity. A control specimen from an unrelated donor should be collected, handled and

shipped at the same time. The acute symptoms of biotinidase deficiency will completely disappear with administration of pharmacological doses of biotin. If given early enough in an infant's life, the prognosis for normal growth and development is good. If children are not detected until neurological damage has occurred, treatment with biotin can reduce further damage but not reverse the damage already done.

### **Screening Practice Considerations**

Detection of the deficiency does not depend on timing or type of feeding because it is an enzyme test. It should therefore be detected on the first specimen unless the infant has been transfused. **ALWAYS OBTAIN A NEWBORN SCREENING SPECIMEN PRIOR TO A TRANSFUSION.** The enzyme is prone to damage if the sample is delayed in shipping or exposed to high temperatures resulting in a possible false positive test. If the blood spot specimen is heavily applied, layered or double spotted, it may be deemed unsatisfactory for testing biotinidase deficiency because of the risk of a false negative result and a repeat specimen will be required.

Prompt confirmatory testing is required even if there is evidence to suggest that one of the situations associated with false positive screens is present. These situations can include heat-damaged specimen. The suspicion of this does not exclude the possibility of disease.

## **CONGENITAL ADRENAL HYPERPLASIA (Salt-Wasting)**

Congenital Adrenal Hyperplasia (classic/severe salt-wasting type due to 21-hydroxylase deficiency) is an inherited disorder of the adrenal cortex. Lack of adequate adrenal cortisol and aldosterone, and increased androgen production. The pituitary gland controls the adrenal glands. When the adrenals are not producing enough of their main hormone “cortisol”, the pituitary pumps out adrenal-stimulating hormone (ACTH). The adrenals make Cortisol, Aldosterone (the salt retaining hormone) and Androgens all from cholesterol. In CAH the enzyme necessary to produce both Cortisol and the salt retaining hormone is missing. This results in insufficient cortisol and aldosterone. The feedback loop tells the pituitary not enough of these are being made, so it produces more adrenal stimulating hormone resulting in an overproduction of androgens, resulting in female virilization of a newborn.

### **Clinical Features**

Salt losers can experience acute dehydration, very low blood pressure, nausea and vomiting, and failure to thrive. The levels of sodium chloride and glucose in the blood fall and the potassium rises. Female infants may be detected clinically by ambiguous genitalia. Males are at highest risk of being undetected without screening. Long term management with pediatric endocrinology is recommended to monitor steroid levels, precocious puberty, rapid early bone growth which may result in short stature. Infants with Congenital Adrenal Hyperplasia are at risk for life-threatening adrenal crises, shock, and death in males and females. Emergency treatment of hospitalization, IV hydrocortisone and IV glucose may be indicated.

### **Lab Test**

The screening test for CAH is an immunoassay for 17-OHP (17-hydroxyprogesterone), and normal reference ranges, cut-offs, and critical cut-offs are based on stratified birthweight ranges, as lower birth weight newborns tend to have higher 17-OHP's on average. While the assay for 17-OHP is considered the state of the art for newborn screening, it tends to have higher false positive rates than for other screened conditions, particularly in premature low birth weight babies. Therefore elevated 17-OHP's reflex to an extracted 17-OHP assay designed to reduce some of the interference or “noise” associated with 17-OHP's in blood spot specimens especially from premature newborns. Because the premature/LBW babies more frequently have abnormal results, the lab algorithm has “preliminary” reports to alert the NICU providers to monitor electrolytes, without unnecessarily triggering confirmatory tests. Specimens collected at less than 24 hours of age will not be tested for CAH.

ABNORMAL TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
17-OHP above the critical cut-off for weight range and baby is > 2500g. POSTIVE report sent, Extracted assay to be run.	(applies to all abnormal results) Salt-wasting CAH -Simple virilizing CAH -False positive -Prematurity or stress	-POSTIVE: <b>Possible neonatal emergency.</b> Consult with pediatric endocrinologist. Notify parents, evaluate newborn immediately and admit to hospital for monitoring. Order confirmatory serum specimen for steroid profile and monitor electrolytes. Ensure steroid profile is tested at laboratory with established neonatal reference ranges. Report lab tests, diagnosis and treatment to NNSP.

ABNORMAL TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
<p>17-OHP above the critical cut-off for weight range but baby is &lt; 2500 g. Preliminary positive reported. Extracted assay run first. If elevated, report amended to Positive. If normal report amended to WNL.</p> <p>-----</p> <p>17-OHP above reference range, but not above the critical cut-off. Extracted 17-OHP above reference range. Inconclusive.</p> <p>-----</p>		<p>-PRELIMINARY POSITIVE: If NICU admission monitor electrolytes, watch for signs/symptoms. If discharged to home contact parents and assess status. Contact pediatric endocrinologist if concerns. If Positive after extracted result is reported, follow steps as above for POSITIVE.</p> <p>-----</p> <p>INCONCLUSIVE. Obtain repeat dried blood spot specimen. Assess infant.</p> <p>-----</p>

### Confirmation & Treatment

For POSITIVE screen results the infant should be assessed immediately. If the baby is not already in the NICU or has been discharged to home, they should be admitted and evaluated. Serum electrolytes should be monitored. IV hydrocortisone, fluids and glucose may be recommended. Contact the pediatric endocrinologist on service. A steroid profile to confirm should be ordered.

### Screening Practice Considerations

NOTE: The screening test for CAH can not detect all forms of CAH (e.g. 11-hydroxylase deficiency), and may not detect all simple virilizing forms.

While abnormal screen results in premature or low birth weight infants may be more common and be associated with stress or prematurity, treatment of POSITIVE screen results should be treated as urgently as for full term newborns.

**Clinical Considerations:** Ambiguous genitalia in females who may appear to be male with non-palpable testes, may be present in a newborn with congenital adrenal hyperplasia, salt –wasting or simple virilizing.

Because less than 24 hour specimens result in an unacceptably high abnormal rate, these specimens are no longer tested for CAH. Therefore obtaining a repeat after 24 hours becomes critical, as does vigilance to watch for the signs and symptoms.

## CONGENITAL PRIMARY HYPOTHYROIDISM

Congenital primary hypothyroidism, or CH or CPH, is the most common disorder detected by newborn screening. It is caused by inadequate production of thyroid hormone. Thyroid hormone is important for the normal functioning of all of the body's organs and is essential for normal brain development. The incidence of congenital primary hypothyroidism is estimated at 1:3,000 to 1:4,000 live births.<sup>1</sup>

The most common causes of CPH are total or partial failure of the thyroid gland to develop (aplasia or hypoplasia), or its development in an abnormal location (an ectopic gland). The screening program is not designed to detect less common causes of hypothyroidism such as those caused by pituitary insufficiency.

### Clinical Features

Deficiency of thyroid hormone in an infant may result in mental and growth retardation if it is not diagnosed and treated early in life. Many infants with CPH may appear clinically normal before 3 months of age, by which time some brain damage has usually occurred.

When symptoms or signs are present, they may include prolonged neonatal jaundice, constipation, lethargy and poor muscle tone, feeding problems, a large tongue, puffy face, large fontanelle, distended abdomen, and umbilical hernia. However, these signs and symptoms are non-specific for CH, are found in fewer than 30% of neonates with CH, and may be present in infants without the condition. Therefore, in the newborn, clinical signs and symptoms are not reliable indicators of CH.

### Laboratory Tests

The primary screening test for thyroxine (T4) level is performed for every newborn. Samples in the lowest 10%ile of T4 results then reflex using a second punch from the same sample and are tested for TSH (thyroid stimulating hormone, or thyrotropin). Specimens collected at less than 24 hours of life will not be tested for T4/TSH due to the expected physiologic surge of the T4 levels. The confirmatory test is a Free T4 and TSH immunoassay performed on a serum sample.

Different combinations of screening test results are possible:

ABNORMAL TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
T <sub>4</sub> low/TSH elevated	<ul style="list-style-type: none"><li>-Hypothyroidism</li><li>-False positive</li><li>-Prematurity (transient hypothyroidism or hypothyroidism of prematurity)</li></ul>	-POSTIVE: Notify parents, and obtain confirmatory serum specimen. Ensure specimen is tested at laboratory with established neonatal reference ranges. Consult with pediatric endocrinologist. Report lab tests, diagnosis and treatment to NNSP.
T <sub>4</sub> low/ TSH normal	<ul style="list-style-type: none"><li>·Thyroid Binding Globulin Deficiency (TBG) deficiency</li><li>·False positive</li><li>·Hypothalamic or Pituitary gland problems with secondary or</li></ul>	For purposes of screening for congenital primary hypothyroidism, this result is normal.



ABNORMAL TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
	tertiary hypothyroidism -Prematurity	
T <sub>4</sub> high/ TSH elevated	-Hyperthyroidism (extraordinarily rare in infants)	For purposes of screening for congenital primary hypothyroidism this result is normal.

### Thyroid Function in Premature Infants

In premature infants, there is a physiological reduction in blood T4 levels. This is generally thought to be due to immaturity of the hypothalamus-pituitary-thyroid axis. This is not due to a low thyroid binding globulin (TBG) and the TSH levels may or may not be elevated. These cases need special observation and follow-up to ensure that the low T4 levels rise to the normal range as the infant matures, but this may take several weeks.

### Confirmation and Treatment

Consultation with a pediatric endocrinologist should be made for confirmation and diagnosis. (See Medical Consultants). Upon notification of a positive screening result the physician should order Serum FT4 and TSH testing to be collected - 200µl of serum specimen. Treatment of congenital primary hypothyroidism is simple and effective. Thyroid hormone in pill form, is crushed, mixed with food and administered once daily. Alternatively, it can be compounded into liquid formulation, but this is only stable for 1 week, and may not suspend uniformly. It is strongly encouraged that infants diagnosed with CPH be followed by a pediatric endocrinologist for exams and blood T4 and TSH levels to assist in regulating the thyroxine dosage, and in managing the “challenge” to rule out transient hypothyroidism. Infants and children should also undergo periodic developmental testing. In Nebraska patients age 0-3 can obtain developmental testing through the Early Development Network (the Early Intervention Part C program)..

### Screening Practice Considerations

Congenital primary hypothyroidism is the most common disorder detected by the program. Detection does not depend on nutritional factors. A blood transfusion may alter the values; therefore THE NEWBORN SCREENING SPECIMEN SHOULD ALWAYS BE COLLECTED PRIOR TO A BLOOD TRANSFUSION.

The normal newborn demonstrates a TSH surge in the first hours of life as an adaptation to the extrauterine environment. To be valid, a specimen must be collected when the infant is at least 24 to 48 hours of age. Less than 24 hour specimens will not be tested for CPH. If an infant’s specimen is collected “early” (less than 24 hours of age) a repeat specimen must be collected within seven (7) days of age but preferably between 24-48 hours of life. Most often early collections are due to premature, low birth weight or sick newborns being admitted to the NICU. In these cases a repeat screen needs to be collected at 48-72 hours.

Prompt confirmatory testing is required even if there is evidence to suggest that one of the situations associated with false positive screens is present. These situations can include early specimen collection and prematurity. The presence of prematurity does not exclude the possibility of disease.

PLEASE NOTE: This screening test is not designed to detect TBG deficiency or causes of hypothyroidism other than congenital primary hypothyroidism. In addition, some infants develop late onset of congenital primary hypothyroidism. If TBG deficiency or other forms of hypothyroidism is suspected, or in the presence of clinical symptoms, appropriate exams and studies are indicated. Therefore, in the presence of clinical symptoms, evaluation for hypothyroidism should be performed despite normal newborn screening results.

## CYSTIC FIBROSIS

The cystic fibrosis transmembrane conductance regulator (CFTR) protein regulates chloride transport that is important for function of lungs, upper respiratory tract, pancreas, liver, sweat glands, and genitourinary tract. CF affects multiple body systems and is associated with progressive damage to respiratory and digestive systems.

CF is one of the most common conditions screened with an incidence rate of approximately 1:3500 white infants, 1:7,000 Hispanic infants, and 1:15,000 black infants.<sup>1</sup> Early diagnosis with newborn screening and comprehensive treatment through an Accredited CF Center is associated with improved growth, reduced hospitalizations and improved pulmonary function and reduced costs.

### CLINICAL FEATURES

Some infants will present with meconium ileus, failure to thrive, recurrent cough, wheezing and chronic abdominal pain. Deficient chloride transport in lungs causes production of abnormally thick mucous leading to airway obstruction, neutrophil dominated inflammation and recurrent and progressive pulmonary infections. Pancreatic insufficiency is found in 80 – 90% of cases. Most males are infertile. More than 1500 mutations in the CFTR gene have been identified, but the  $\Delta F508$  is associated with 70% of patients from European ancestry

### LAB TEST

The primary screening test for CF is an assay for Immuno-reactive trypsinogen (IRT). To maximize the likelihood of picking up most cases of CF and minimize the burden of too many false positives on the screening system, all IRT's greater than 90 reflex to DNA analysis for the  $\Delta F508$  mutation. If no  $\Delta F508$  is found, a repeat dried blood spot is recommended. If one copy of  $\Delta F508$  is found the test reflexes again on a new punch from the initial or same specimen to look at a 39 + 4 CFTR mutation panel of some of the more commonly found mutations. If one or two mutations are found, referral to an Accredited CF Center and sweat testing is recommended. Specimens collected at less than 24 hours of life will not be tested for CF due to the high rate of inconclusive reports due to commonly elevated IRT levels in < 24 hour specimens.

ABNORMAL TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
IRT > 90, no $\Delta F508$ Inconclusive	Carrier of Cystic Fibrosis CF related metabolic syndrome CF False positive Asphyxia Trisomy 13, 18, 21 Gastrointestinal abnormalities Prematurity Elevated liver enzymes Specimen collected < 24 °	Obtain a repeat dried blood spot specimen. (If repeat testing results in IRT still > 90, the test reflexes to the 39 + 4 mutation panel).
IRT > 90, one copy $\Delta F508$	Carrier of Cystic Fibrosis	Recommend referral to CF Center and sweat

ABNORMAL TEST RESULTS	LIKELY CAUSES	Health Care Provider Actions
Inconclusive	CF related metabolic syndrome	testing. Genetic counseling for parents and consideration of further genetic testing.
IRT > 90, two copies ΔF508 or one copy ΔF508 with another mutation POSITIVE	Cystic Fibrosis CF related metabolic syndrome	Recommend referral to CF Center and sweat testing,

## CONFIRMATION AND TREATMENT

Any newborn with a persistently elevated IRT (on repeat) or elevated IRT with one or more mutations should be referred to an Accredited CF Center for evaluation, sweat testing and diagnosis. Diagnosed patients should receive comprehensive care including genetic counseling, pulmonology, nutrition, radiology and infectious disease services.

## SPECIAL CONSIDERATIONS

Meconium Ileus. If a baby is found to have meconium ileus (not meconium stain) or other bowel obstruction, the newborn screening program and laboratory should be notified immediately. The IRT assay for newborns with MI or other bowel obstruction is known to often have low levels. A normal or low IRT would not trigger reflex testing of the ΔF508 mutation. Therefore because MI or other bowel obstruction is often associated with severe cystic fibrosis, notifying the newborn screening laboratory will trigger reflex testing to immediately run the 39 + 4 CFTR mutation panel.

It is especially important to recognize that screening for Cystic Fibrosis has somewhat lower sensitivity than other conditions screened. In the first 5 years of screening for CF in Nebraska three cases of CF were detected clinically that were not identified by the screen. In response to one of those cases, the screening algorithm has been modified. Health Care Providers should consider evaluation for cystic fibrosis in patients presenting with symptoms, even if newborn screening results were normal. In addition each State NBS Program has different screening algorithms with different levels of sensitivity and specificity, so if the infant or child was born outside Nebraska, a different screening algorithm may have been used.

There are numerous reasons for elevated IRT in newborns, including specimen collection at < 24°, asphyxia, stress, Trisomies 13, 18, 21, septicemia, congenital cytomegalovirus, obstructive liver disease, biliary atresia. African American babies tend to have higher numbers of elevated IRT's but a lower incidence of diagnosed cystic fibrosis.

## GALACTOSEMIA

Galactosemia is an autosomal recessive inherited metabolic disorder. Abnormal galactose metabolism can be caused by the deficiency of any of the three enzymes of the galactose catabolic pathway: galactose-1-phosphate uridyl transferase, galactokinase, or UDP-galactose-4-epimerase. Clinically, deficiency of galactose-1-phosphate uridyl transferase has become synonymous with classical galactosemia. Because Nebraska's screening test is for galactose and GALT (transferase), the targeted condition for screening is galactosemia associated with the deficient enzyme galactose-1-phosphate uridyl transferase. Classic Galactosemia (transferase) occurs in approximately 1 in 47,000 infants.

Galactose is a component of lactose, the principle carbohydrate of mammalian milk and most non-soy commercial infant formulas. Lactose is hydrolyzed to glucose and galactose in the intestine. The body cannot break down the galactose without the proper enzymes. The galactose builds up in the body and causes cellular damage and even death. The incidence of galactosemia is estimated at 1:60,000 to 1:80,000 live births.<sup>1</sup>

### Clinical Features

The severe form of this disease is due to almost total deficiency of galactose-1-phosphate uridyl transferase enzyme activity in all cells of the body. The early clinical features of severe galactosemia include liver dysfunction manifest as jaundice and hypoglycemia; neurological findings of irritability and seizures; and gastrointestinal findings of poor feeding, failure to thrive, vomiting, and diarrhea. Death may result from gram-negative sepsis within one to two weeks of birth. If the infant is untreated and survives the neonatal period, cataracts, cirrhosis, Fanconi syndrome, and neurological damage are usual developments.

There are several genetic variants characterized by a less severe reduction in enzyme activity (e.g. Duarte Variant). Most of these cases are asymptomatic and are detected because of a persisting abnormality in the enzyme test. However, some of these cases may benefit from dietary therapy. For this reason, infants with elevated galactose and normal GALT require further testing and should be evaluated by a pediatric metabolic specialist.

### Laboratory Test

The Beutler and Baluda qualitative screening test is used. Dried blood spots are incubated with galactose-1-phosphate, uridine diphosphoglucose, and NADP. In the presence of galactose-1-phosphate uridyl transferase, a sequence of enzyme reactions proceeds with generation of NADPH which is detected by fluorescence or colorimetrically.

LAB RESULTS	LIKELY CAUSES	Health Care Provider Actions
Elevated Galactose, Low Uridyl transferase (POSITIVE)	-Severe galactosemia -Improperly collected sample (heat damage or transit delay) -Mild galactosemia variant -Other enzyme defect in RBC's -False positive	<b>POSITIVE: Potential Neonatal Emergency.</b> Consult with pediatric metabolic specialist. Contact parents and obtain serum confirmatory test for galactose-1-phosphate uridyl-transferase. Interrupt breast or mammalian milk formula and initiate powder-based soy formula. Report lab

LAB RESULTS	LIKELY CAUSES	Health Care Provider Actions
Elevated Galactose, Normal GALT (Inconclusive)	(same as above)	tests, diagnosis and treatment to NNSP.  Inconclusive: Contact parents and obtain repeat dried blood spot specimen testing. Most often repeats will be normal. If galactose is still elevated on repeat, refer to pediatric metabolic specialist.

## Confirmation and Treatment

Consultation with a pediatric metabolic specialist should be made for confirmation and diagnosis (see Medical Consultants). Upon notification of a positive screening result the physician should direct the family to interrupt breast feeding and stop the feeding of lactose or galactose containing formulas. A powder-based soy formula is recommended. The physician should collect or cause to be collected 2 ml. of whole blood, lithium heparnized (green top) and order a confirmatory test for galactose-1-phosphate uridyl-transferase. The galactosemia syndromes are treated by a rigid dietary exclusion of all galactose. Exclusion of milk and milk products alone does not constitute a galactose-restricted diet, as galactose is found in other foods as well. A galactose-free diet requires close supervision and monitoring. Referral to a pediatric metabolic specialist is strongly recommended.

## Screening Practice Considerations

The Beutler test should be abnormal in all severe (classical) galactosemic infants - even if the specimen is collected before lactose is ingested - unless the infant has been transfused. ALWAYS OBTAIN A NEWBORN SCREENING SPECIMEN PRIOR TO A TRANSFUSION.

Galactosemia can kill quickly. It should be considered in any infant with non-glucose reducing substances in the urine. If galactosemia is suspected, immediately consult with a pediatric metabolic physician. In some cases the specialist may recommend testing urine for reducing sugars, in addition to the confirmatory test.

NOTE: Galactose reacts with "Clinitest" and with most blood "glucose" methods, such as Somogyi-Nelson, etc., which measure total blood sugars (but not with glucose oxidase methods, i.e. Clinistix). Galactosemia produces a positive Clinitest and a negative Clinistix result.

Prompt confirmatory testing is required even if there is evidence to suggest that a situation associated with false positive screens is present. One of these situations can include heat-damaged specimen. The presence of this does not exclude the possibility of disease.

If screening test results are positive for galatosemia, the physician shall take the child off milk and then collect or cause to be collected a new specimen for confirmatory tests. A powder based soy formula is recommended.

## HEMOGLOBINOPATHIES

Most clinically significant hemoglobinopathies are inherited defects of the beta ( $\beta$ ) globin chain of adult hemoglobin. Red blood cells of newborns have a predominance of fetal hemoglobin which does not contain  $\beta$  globin. For this reason, clinical signs and symptoms of  $\beta$  globin abnormalities are usually not apparent at birth but become evident as adult hemoglobin replaces fetal hemoglobin.

Most of the hemoglobinopathies detected by newborn screening are the result of single amino acid substitutions in the  $\beta$  globin and are inherited as autosomal recessive disorders. Persons with two abnormal  $\beta$  globin genes (homozygotes or double heterozygotes) have “disease.” Individuals with one abnormal  $\beta$  globin gene (heterozygotes) have “trait” and are carriers for “disease.” That is, individuals can pass on “trait” or potential for “disease” to offspring.

Thalassemias are caused by the decreased synthesis of the globin chains. Infants with  $\alpha$  thalassemia may be identified by newborn screening because Barts hemoglobin (composed of four gamma chains) is readily detected.  $\beta$  thalassemia genes may interact with genes for  $\beta$  globin variants and produce serious hemoglobinopathies, but may not be detectable at birth..

The frequency of hemoglobinopathies varies among ethnic groups in the United States. Sick cell hemoglobin is found in higher frequencies in descendants of people from Africa, Italy, Greece, Turkey, Arabia, and India. Hemoglobin C occurs predominantly (but not exclusively) among descendants of people from central and western Africa. Hemoglobin E is common in persons of Southeast Asian ancestry. Many thalassemia genes originated in west and central Africa, Italy, Greece, Asia, Africa, and the Pacific Islands. Overall sick cell disease occur in 1:2500 to 1:2000 US newborns and is estimated to occur in 1:346 black infants, and in 1:1114 Hispanic infants in the Eastern US.<sup>1</sup>

### Clinical Features

Sickle cell disease occurs in persons homozygous for the sickle gene (sickle cell anemia), in compound heterozygotes for sickle hemoglobin and hemoglobin C (hemoglobin SC disease), and in compound heterozygotes for sickle hemoglobin and  $\beta$  thalassemia (sickle- $\beta$  thalassemia).

Infants with sickle cell disease may present with dactylitis, fever and sepsis, jaundice, anemia, or splenic sequestration (which may be life threatening in a small child) at any time after a few months of age. Other symptoms may occur as the disease progresses, such as recurrent pain, acute chest syndrome, and stroke. These disorders vary in severity, and some clinical features are not present in all affected individuals.

In other hemoglobin diseases, clinical features are influenced by the type of hemoglobin variant. Homozygous hemoglobin C or hemoglobin E show only mild hemolytic anemia. Persons with thalassemias have varying degrees of microcytic hypochromic anemia, and the severe forms (E- $\beta$  thalassemia) may have hemolysis and be transfusion dependent.

Comprehensive medical care coordinated with a pediatric hematologist or pediatrician specializing in hemoglobinopathies reduces mortality in infancy and early childhood.

Most hemoglobin traits (heterozygotes) are associated with few or no clinical problems in childhood. Thus, the value of trait or carrier detection is the opportunity to educate families, to test other family members, and to provide genetic counseling.

## Laboratory Tests

Screening is performed by isoelectric focusing (IEF) of a hemolyate prepared from a dried blood spot. Hemoglobin “bands” are identified by the migration in the electrophoretic field. The newborn screening test is highly sensitive and specific. When screen results are positive for FS, FSA, FC, FSC, or FE a new punch from the same specimen is tested at the screening laboratory for DNA associated with hemoglobins S, C and E and alpha & beta thalassemia. The confirmatory test is isoelectric focusing hemoglobin electrophoresis.

<b>*TEST RESULTS</b>	<b>LIKELY CAUSES</b>	<b>Health Care Provider Actions</b>
FA	-Normal	-None, report result to parents
FAS	-Sickle cell trait -Sickle cell anemia after blood transfusion	-Confirmatory testing as above -Offer genetic counseling to family -Report lab results and diagnosis to NNSP
FSA	-S- $\beta$ thalassemia	-Confirmatory testing as above -Offer genetic counseling to family -Referral to pediatric subspecialist -Report lab results, diagnosis and treatment to NNSP
FAC	-Hemoglobin C trait	-Confirmatory testing as above -Offer genetic counseling to family -Report lab results and diagnosis to NNSP
FAV	-Possibilities include many hemoglobin traits	-Confirmatory testing as above -Offer genetic counseling to family -Report lab results and diagnosis to NNSP
FA + Barts $\ddagger$	-Possibilities include hemoglobin Barts which is indicative of $\alpha$ thalassemia  -Artifact	-Confirmatory testing as above -Offer genetic counseling to family -Consider referral to pediatric subspecialist in hemoglobinopathies -Report lab results, diagnosis and treatment to NNSP
F only	-Premature infant - $\beta$ thalassemia major -Hereditary persistent fetal hemoglobin	-Confirmatory testing as above -Offer genetic counseling to family -Referral to pediatric subspecialist in hemoglobinopathies -Report lab results, diagnosis and treatment to NNSP
AF Predominant A	-Transfused infant -Normal matured infant	-If this was on initial specimen, verify transfusion. If no screen before transfusion,



<b>*TEST RESULTS</b>	<b>LIKELY CAUSES</b>	<b>Health Care Provider Actions</b>
		re-screen at 90-120 days of age.
FV (no A)	-Possibilities include homozygous Variant or Variant beta thalassemia	-Confirmatory testing as above -Offer genetic counseling to family -Referral to pediatric subspecialist in hemoglobinopathies -Report lab results, diagnosis and treatment to NNSP
FS (no A)	-Sickle cell anemia -Sickle- $\beta$ thalassemia	-Confirmatory testing as above -Offer genetic counseling to family -Referral to pediatric subspecialist -Report lab results, diagnosis and treatment to NNSP
FSC (no A)	<sup>4</sup> -Sickle-hemoglobin C disease	-Confirmatory testing as above -Offer genetic counseling to family -Referral to pediatric subspecialist -Report lab results, diagnosis and treatment to NNSP
FC (no A)	-Homozygous hemoglobin C	-Confirmatory testing as above -Offer genetic counseling to family -Referral to pediatric subspecialist -Report lab results, diagnosis and treatment to NNSP

\* Hemoglobins are reported in order of quantity (e.g., FAS = F>A>S).

F = fetal hemoglobin

A = adult hemoglobin

S = sickle hemoglobin

V = unidentified migrating band variant

C = hemoglobin C

‡ Barts hemoglobin is a fast migrating band that is present at birth in infants with  $\alpha$  thalassemia and generally disappears by 4 months of age. Thus, infants with an unidentified fast band on the screening tests and with a normal confirmatory test should be suspected of having  $\alpha$  thalassemia, especially if they have microcytosis and are of Asian, Mediterranean, or African ancestry. However, the absence of an unidentified fast band on the screening test does not exclude the possibility of  $\alpha$  thalassemia.

## Confirmation and Treatment

Consultation with a pediatric hematologist should be made for confirmation and diagnosis for clinically significant hemoglobinopathies. (see Medical Consultants). Upon notification of a positive hemoglobinopathy screening result the physician should collect or cause to be collected 0.5 ml (purple top) EDTA whole blood and order a confirmatory hemoglobinopathy test (isoelectric focusing hemoglobin electrophoresis). It is important to obtain confirmation results and a diagnosis by 2 months of age for some clinically significant hemoglobinopathies (such as sickle cell disease) in order to initiate prophylactic treatment. Treatment for hemoglobin diseases is determined by the type and severity of the hemoglobin disorder.

## **Screening Practice Considerations**

The laboratory tests should detect most abnormal hemoglobin subtypes, even if the specimen is collected before 24 hours of age, unless the infant has had an exchange transfusion. Blood transfusions may cause false negative results. **ALWAYS OBTAIN A NEWBORN SCREENING SPECIMEN PRIOR TO A TRANSFUSION.**

The primary purpose of hemoglobinopathy screening is the identification of infants with sickle cell diseases for whom early intervention has been shown to markedly reduce morbidity and mortality. The screening test is not diagnostic and confirmation of all abnormal results should be done.

The most commonly identified abnormal phenotype through newborn screening is FAS which is presumably positive for sickle trait. Parents of infants with sickle cell trait have a chance of having an infant with sickle cell disease if both parents carry the gene for sickle hemoglobin. Further testing of both parents combined with genetic counseling can apprise parents of their chances in future pregnancies of having children affected with clinically significant disease. Every infant with a presumptive positive hemoglobinopathy screening result, must have confirmatory testing done in a timely manner.

Hemoglobinopathies are complex disorders, and practitioners are strongly encouraged to consult a pediatric hematologist and follow-up resources for more information concerning abnormal screening results and appropriate follow-up and treatment.

## **MCAD**

### **Medium Chain Acyl Co-A Dehydrogenase Deficiency\***

\*This practitioner's manual describes one condition from each of the 3 classifications (AA's, FAO's, and OA's) of disorders screened by tandem mass spectrometry (MS/MS). MCAD is an example of a Fatty Acid Oxidation (FAO) disorder detectable by this screen. While other FAO's may have similar presentations and characteristics, each are distinct. For more information on each condition we recommend referencing the ACMG's ACT sheets at <http://www.ACMG.net> , Genetic Home Reference at <http://ghr.nlm.nih.gov> and tables included in "Toward a Uniform Newborn Screening Panel" Genetics in Medicine, Vol 8, Supp 1, May 2006

MCAD apparently is the most common of the 10 disorders of mitochondrial fatty acid oxidation identified to date. Prior to universal screening MCAD was later found to be the cause of death in infants and children in some cases of SIDS and Reye syndrome. MCAD is found almost exclusively in individuals from northwestern European origin, with incidence approximately 1:15,011. Classic presentation includes episodes of hypoketotic hypoglycemia triggered by fasting because of the inability to break down fats to ketone bodies for an energy source. Medium chain acylcarnitines accumulate in the mitochondria inhibiting mitochondrial  $\beta$ -oxidation. Fat accumulates in the liver during acute episodes. Secondary to the accumulation of fatty acids in the central nervous system, encephalopathy and cerebral edema occur. The combination of hypoglycemia and toxic effects of the fatty acids or metabolites contributes to coma.

#### **Clinical Features**

During periods of fasting, (e.g. gastrointestinal or upper intestinal viral infection, rarely neonatally when full breast feedings not yet established, or extended periods of time between feedings) episodes of vomiting and lethargy, may be followed by seizures, coma and death. Infants who survive the metabolic crisis may have developmental disabilities, speech and language delay, behavioral problems, ADHD, proximal muscle weakness, chronic seizure disorder, cerebral palsy and failure to thrive. Newborns appear normal at birth. Avoidance of fasting more than 4 hours during neonatal period, IV glucose and carnitine during times of viral illness and monitoring by a metabolic specialist can avoid the devastating outcomes. Some patients may receive supplemental carnitine.

#### **Maternal HELLP syndrome**

A higher suspicion of MCAD or other fatty acid oxidation disorders should occur when the mother is known to have HELLP syndrome (Hemolytic anemia, elevated liver enzymes and low platelet count) or acute fatty liver disease.

#### **Laboratory Test**

The screening test for MCAD is MS/MS analysis of acylcarnitines. Elevations in the C8 (octanoylcarnitine), or C8 plus elevated ratios of C8:C6 and C8:C10 are abnormal for MCAD. As with all MS/MS results the interpretation will be reported out as slightly elevated, abnormal or substantially elevated. Specimens with abnormal or substantially elevated C8 results reflex to DNA for the most common mutation known to be associated with MCAD, A985G.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Mild elevation of C8  ----- Abnormal level of C8 and or C8:C6 or C8:C10  ----- Substantial elevation of C8 and or C8:C6 or C8:C10 possibly with A986G mutation	MCAD False positive	-Obtain repeat dried blood spot specimen testing within 48 hours  ----- -Obtain repeat dried blood spot specimen testing immediately  ----- -Obtain quantitative plasma acylcarnitines, urine organic acids. -Consult with and refer to pediatric metabolic specialist -Report confirmatory lab results, diagnosis and treatment to NNSP

## Confirmation and Treatment

Consultation with a pediatric metabolic specialist should be made for confirmation and diagnosis (see Medical Consultants). Upon notification of a positive screening result (substantial elevation), the physician should order quantitative plasma acylcarnitines and urine organic acids. Parents should be counseled to not allow the infant to go more than four (4) hours between feedings. The child should be admitted to the hospital for signs/symptoms of viral illness. With early and proper treatment the devastating outcomes can be avoided.

## Special Considerations

MCAD is a relatively easy, inexpensive and effectively treated condition by preventive measures. MCAD like all conditions presents as a spectrum of disease, even within families with multiple members affected. One example of a family with several affected children had one child die from the first episode before 2 years of age, and other children as old as 10 years never having had an episode.<sup>1</sup> The genotype/phenotype correlation is not yet well understood, nor are modifying factors.

## **PHENYLKETONURIA (PKU)**

\*This practitioner's manual describes one condition from each of the 3 classifications (AA's, FAO's, and OA's) of disorders screened by tandem mass spectrometry (MS/MS). PKU is an example of an Amino Acid (AA) disorder detectable by this screen. While other AA's may have some common potential outcomes, each are very distinct. For more information on each condition we recommend referencing the ACMG's ACT sheets at <http://www.ACMG.net>, Genetic Home Reference at <http://ghr.nlm.nih.gov> and tables included in "Toward a Uniform Newborn Screening Panel" Genetics in Medicine, Vol 8, Supp 1, May 2006

Phenylketonuria, or PKU, is caused by a recessively inherited enzyme defect in which the body cannot properly use the amino acid phenylalanine. All other metabolic processes are intact but phenylalanine, which comes from all dietary protein, accumulates in the blood. Excess phenylalanine cannot be converted to tyrosine due to a lack of phenylalanine hydroxylase, the enzyme that catalyzes the conversion of phenylalanine to tyrosine. Phenylalanine accumulates in the body and causes damage. Overall, PKU occurs in about 1 in 13,500 to 1:19,000 U.S. live births.<sup>1</sup> With appropriate treatment, the risk of any of these conditions is substantially reduced.

- Classical phenylketonuria is a disorder in which the blood phenylalanine, or phe, rises above 20 mg/dL on a normal diet (normal blood phe is less than 2.0 mg/dL). Without treatment, nearly all affected individuals develop severe mental retardation. Other symptoms include severe mental deficiency, microcephaly, eczematous or oily skin, cerebral palsy, convulsions, dysphasia, hyperactivity with purposeless movements, autistic-like behavior, and an abnormal EEG. In infants, vomiting may mimic pyloric stenosis. The skin and hair are usually fair, the eyes may be blue and a "mousey" odor of the baby's urine is frequent. The smell arises from phenylacetic acid.

- Hyperphenylalaninemia refers to any consistent elevation of phe levels, including classical PKU. If cases of classical PKU are excluded, this includes blood phe levels less than 20 mg/dL. These may be caused by liver damage, transient tyrosinemia of prematurity, mutation of the phenylalanine hydroxylase gene, disorders of cofactor synthesis or regeneration, or maternal PKU. In these cases, mental retardation may or may not be present. Blood levels may remain elevated throughout life or may gradually fall towards normal. In infancy, these patients can mimic the severe PKU condition, and even in mild cases there seems to be an increased risk of the maternal PKU syndrome, as described below.

- Much rarer causes of elevated phenylalanine are caused by defects of bipterin metabolism. Blood phenylalanine levels are variable. These patients have progressive neurological damage with seizures and steady deterioration which becomes noticeable sometime between 6 and 20 months of age despite early treatment with a low phenylalanine diet. In view of the severity of this group of diseases, all infants with persistently abnormal levels of phenylalanine should be tested by special blood and urine tests for bipterin abnormalities.

The cause of hyperphenylalaninemia must be determined if proper treatment is to be provided. Definitive tests can differentiate these variant forms of PKU. Treatment by dietary restriction alone is inadequate for a tetrahydrobiopterin cofactor defect. The cofactor is necessary not only for normal activity of phenylalanine hydroxylase, but also for activity of the tyrosine and tryptophan hydroxylase which are involved in serotonin and dopamine synthesis.

## Maternal PKU

Pregnancy presents a special problem with PKU and other forms of hyperphenylalaninemia, as high blood levels of phe are teratogenic to the fetus. This is important in the context of newborn screening because early testing of an infant born to a mother with PKU or hyperphenylalaninemia can reflect the mother's phe levels. A positive test on a newborn who then has a normal repeat test, especially if the baby has growth retardation, microcephaly, or malformations, should raise the possibility of maternal PKU. These infants usually have a transient elevation of phenylalanine (3-20 mg/dL) reverting to normal within 24 hours. If maternal PKU is suspected, the mother should be appropriately tested and counseled.

A medically supervised phenylalanine restricted diet begun before conception with phenylalanine levels maintained within a safe range throughout pregnancy may prevent damage to the fetus.

## Laboratory Test

The screening test for PKU is done by tandem mass spectrometry to determine the molecular mass of the phenylalanine and phenylalanine:tyrosine ratios. Values will be provided on the laboratory report and classified as mild elevation, abnormal or substantially elevated.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Mild elevation of Phenylalanine  ----- Abnormal level of phenylalanine  ----- Substantial elevation of phenylalanine possibly with elevated phe/tyr ratio	(for all abnormal findings) -PKU -Mild hyperphenylalanemia -False positive -Maternal PKU	-Obtain repeat dried blood spot specimen testing within 48 hours  ----- -Obtain repeat dried blood spot specimen testing immediately  ----- -Obtain quantitative plasma amino acids -Consult with and refer to pediatric metabolic specialist -Report confirmatory lab results, diagnosis and treatment to NNSP

## Confirmation and Treatment

Consultation with a pediatric metabolic specialist should be made for confirmation and diagnosis (see Medical Consultants). Upon notification of a positive screening result (substantial elevation), the physician should collect or cause to be collected 0.5 ml heparinized plasma for quantitative testing of phenylalanine and tyrosine. Serum is also acceptable. With early and proper treatment, mental retardation is usually preventable. Treatment should be started as soon after birth as possible in any infant with phenylalanine levels over 8 mg/dL and should be continued for life.

Frequent monitoring and diet adjustment is required, especially in the first weeks, because infants with variant forms of hyperphenylalaninemia may be indistinguishable from true PKU and improper nutritional therapy

can produce brain damage or be fatal. Specific formulas for the diet management and expert nutritional supervision are provided through University of Nebraska Medical Center and Children's Hospital Metabolic Clinics.

If treatment is not started for some weeks, the results are more variable and the I.Q. tends to be lower. Patients who are not treated until after six months of age may show some improvement in I.Q., although they are likely to remain retarded. Older patients usually show little change in I.Q. with treatment, but a low phenylalanine diet may help to control serious behavior problems and other manifestations of untreated PKU.

### **Screening Practice Considerations**

Plasma phenylalanine is not detectably elevated in cord blood. It starts rising within 24 hours after birth and reaches 20 mg/dL or more within a few days. The screening test is often abnormal within 24 hours after birth and almost uniformly abnormal beyond 24 hours of birth in those infants with PKU.

The phe level of affected infants rises gradually after birth with little, if any, effect of the amount of protein ingested by the infant. The infant must be at least 24 hours of age to reliably detect PKU. Those with milder forms of hyperphenylalaninemia may need to be older to develop abnormal tests.

If an infant is tested "early" (less than 24 hours of age) a repeat test must be performed by seven (7) days of age (preferably at 24-48 hours), regardless of prior test results, as treatment started after 1 month of age may be less than optimal. If an infant is tested at less than 24 hours of age because of NICU admission, the repeat screen should be done at 48-72 hours of life.

The reliability of the screening result does NOT depend on the amount of feedings the newborn has had.

Dialysis or transfusion may temporarily lower phe levels. **ALWAYS OBTAIN A NEWBORN SCREENING SPECIMEN PRIOR TO A TRANSFUSION.** Children who develop retardation before one year of age should undergo testing for PKU even if a negative screening test is documented.

Prompt confirmatory testing is required even if there is evidence to suggest that one of the situations associated with false positive screens is present. These situations can include inadequate specimen collection, or hyperalimentation. The presence of any of these does not exclude the possibility of disease.

While infants receiving hyperalimentation may have false positive results for PKU, it is generally believed the tandem mass spectrometry interpretation can distinguish those infants at higher risk of having PKU from those on hyperalimentation. This does not apply to other amino acid conditions screened, that are less likely to be able to be distinguished from elevations due to hyperalimentation.



### **C3**

## **PROPIONIC ACIDEMIA (PA)**

### **OR**

## **MEHTYLMALONIC ACIDEMIA (MMA)**

\*This practitioner's manual describes one condition from each of the 3 classifications (AA's, FAO's, and OA's) of disorders screened by tandem mass spectrometry (MS/MS). PA and MMA are example of Organic Acidemia's (OA's) disorder detectable by this screen. PA/MMA provides a good example of how common analyte markers may indicate be trigger differential diagnosis for more than one condition. Other OA's may have some common potential outcomes but each are very distinct. For more information on each condition we recommend referencing the ACMG's ACT sheets at <http://www.ACMG.net>, Genetic Home Reference at <http://ghr.nlm.nih.gov> and tables included in "Toward a Uniform Newborn Screening Panel" Genetics in Medicine, Vol 8, Supp 1, May 2006

PA is caused by a defect in propionyl-CoA carboxylase which converts propionyl-CoA to methylmalonyl-CoA. MMA results from a defect in methylmalonyl-CoA mutase which converts methylmalonyl-CoA to succinyl-CoA or from lack of the required B12 cofactor for methylmalonyl-CoA mutase (cobalamin A, B, C, D, and F).

The condition description below is excerpted from "Genetic Home Reference" <http://ghr.nlm.nih.gov> for propionic acidemia and methylmalonic acidemia.

Propionic acidemia and methylmalonic acidemia are inherited disorders in which the body is unable to process certain parts of proteins and lipids (fats) properly. They are classified as organic acid disorders, which are conditions that lead to an abnormal buildup of particular acids known as organic acids. In PA the *PCCA* and *PCCB* genes provide instructions for making two parts (subunits) of an enzyme called propionyl-CoA carboxylase. This enzyme plays a role in the normal breakdown of proteins. Specifically, it helps process several amino acids. Propionyl-CoA carboxylase also helps break down certain types of fat and cholesterol in the body. Mutations in the *PCCA* or *PCCB* gene disrupt the function of the enzyme and prevent the normal breakdown of these molecules. As a result, a substance called propionyl-CoA and other potentially harmful compounds can build up to toxic levels in the body. This buildup damages the brain and nervous system, causing the serious health problems associated with propionic acidemia.<sup>2</sup>

In most cases, the features of propionic acidemia become apparent within a few days after birth. The initial symptoms include poor feeding, vomiting, loss of appetite, weak muscle tone (hypotonia), and lack of energy (lethargy). These symptoms sometimes progress to more serious medical problems, including heart abnormalities, seizures, coma, and possibly death. Less commonly, the signs and symptoms of propionic acidemia appear during childhood and may come and go over time. Some affected children experience intellectual disability or delayed development. In children with this later-onset form of the condition, episodes of more serious health problems can be triggered by prolonged periods without food (fasting), fever, or infections. Propionic acidemia affects about 1 in 100,000 people in the United States

In MMA the effects which usually appear in early infancy, vary from mild to life-threatening. Affected infants experience vomiting, dehydration, weak muscle tone (hypotonia), excessive tiredness (lethargy), and failure to

gain weight and grow at the expected rate (failure to thrive). Long-term complications can include feeding problems, intellectual disability, chronic kidney disease, and inflammation of the pancreas (pancreatitis). Without treatment, this disorder can lead to coma and death in some cases. MMA occurs in an estimated 1 in 50,000-100,000 people.

About half of methylmalonic acidemia cases are caused by mutations in the *MUT* gene. This gene provides instructions for making an enzyme called methylmalonyl CoA mutase. This enzyme works with vitamin B12 to break down several protein building blocks (amino acids), certain lipids, and cholesterol. Mutations in the *MUT* gene alter the enzyme's structure or reduce the amount of the enzyme, which prevents these molecules from being broken down properly. As a result, a substance called methylmalonyl CoA and other potentially toxic compounds can accumulate in the body's organs and tissues, causing the signs and symptoms of methylmalonic acidemia.

Mutations in the *MUT* gene that prevent the production of any functional enzyme result in a form of the condition designated mut0. Mut0 is the most severe form of methylmalonic acidemia and has the poorest outcome. Mutations that change the structure of methylmalonyl CoA mutase but do not eliminate its activity cause a form of the condition designated mut-. The mut- form is typically less severe, with more variable symptoms than the mut0 form. Other cases of methylmalonic acidemia are caused by mutations in the *MMAA* or *MMAB* genes. To function properly, methylmalonyl CoA mutase must work together with the proteins produced from these genes. Mutations that affect either of these proteins can impair the activity of methylmalonyl CoA mutase, leading to methylmalonic acidemia. The long term effects of methylmalonic acidemia depend on which gene is mutated and how the enzyme is affected by the mutation. It is likely that other unidentified genes also cause methylmalonic acidemia.

## CLINICAL CONSIDERATIONS

Patients with PA and severe cases of MMA typically present in the neonate with metabolic ketoacidosis, dehydration, hyperammonemia, ketonuria, vomiting, hypoglycemia, and failure to thrive. Long-term complications are common, early treatment may be lifesaving and continued treatment may be beneficial.

## LAB TESTS

The screening test for PA/MMA is done by tandem mass spectrometry to determine the molecular mass of the C3 and ratios of C3:C2 and C3:C16. Values will be provided on the laboratory report and classified as mild elevation, abnormal or substantially elevated.

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
Mild elevation of C3 (propionylcarnitine) -----	(for all abnormal findings) -Propionic Acidemia -Methylmalonic Acidemia (mutase)	-Obtain repeat dried blood spot specimen testing within 48 hours -----
Abnormal level of C3 and ratios of C3:C2 and or C3:C16 -----	-Methylmalonic Acidemia (Cbl A or B) -Maternal deficiency of B <sub>12</sub>	-Obtain repeat dried blood spot specimen testing immediately -----
Substantial elevation of C3 possibly with elevations of C3:C2	-False positive	-Contact family and assess newborn for poor feeding vomiting, lethargy, tachypnea -Obtain ketones, plasma acylcarnitines, plasma amino acids, urine organic acids for differential

ABNORMAL TEST RESULT	LIKELY CAUSES	Health Care Provider Actions
and or C3:C16		diagnosis -Consult with and refer to pediatric metabolic specialist -Report confirmatory lab results, dx/tx to NNSP

# **SCREENING PRACTICES**

## **PROPER TIME FOR TESTING**

All newborns should have their screening specimen collected between 24-48 hours of life.

Exceptions:

Newborns being discharged even if < 24 hours of age, should have an initial screen collected. A repeat is required after 24 hours of age, within 48 hours.

Newborns being transferred to a NICU, even if < 24 hours of age. A specimen is required to be collected prior to transfer.

The optimal time for obtaining specimens varies for the different conditions. In conditions such as MCAD the first days after birth while catabolic may be ideal for identification until in a well-fed state. In conditions such as PKU, accumulation of phenylalanine in the plasma does not occur until after birth when the production rate of phenylalanine exceeds the infant's capacity to metabolize or excrete it. Similarly, because there is a normal TSH surge in infants immediately following birth, there would be many false positive results for congenital primary hypothyroidism in specimens collected prior to 24 hours. In addition IRT's are often elevated in < 24 hour specimens.

The trend towards early discharge from hospitals and the increase in home births complicates newborn screening. It is sometimes difficult to decide whether the risks of missing a case of PKU by testing too early outweighs the risks of delaying testing until after the infant is discharged home or of missing timely screening altogether due to failure to obtain a post-discharge specimen. Infants with untreated galactosemia usually die within a week or two of birth due to the severe metabolic consequences of their disease. The American Academy of Pediatrics recommends that all infants tested before 24 hours of age should be retested to avoid missing PKU.

## **Program Requirements**

Nebraska regulations (see appendix) require a newborn screening specimen to be collected between 24 and 48 hours of age, or immediately prior to transfer, transfusion or discharge, whichever occurs first.

Nebraska also requires that any infant whose newborn screening specimen was collected prior to 24 hours of age have another specimen collected by seven (7) days of age (preferably at 24-48 hours of age), regardless of prior test results. Specimens collected at less than 24 hours of age are not tested for CAH, CPH or CF, and may be unreliable for detecting aminoacidopathies. See special requirements for premature, low birth weight and sick newborns admitted to neonatal intensive care units.

## **SPECIAL CONSIDERATIONS OF NEWBORN SCREENING**

### **NEWBORN SCREENING OF AN INFANT WHO EXHIBITS CLINICAL SIGNS AND SYMPTOMS**

The newborn screening test, like any laboratory test, may have false positives and false negatives. If signs and symptoms of one of the disorders are clinically evident, the physician should proceed to diagnostic testing, pending the results of the screen or in spite of the results of the screen.

If the test results are pending for galactosemia (in which the metabolite accumulation can be very dangerous), proceed as if the infant has the condition. Contact a pediatric metabolic physician for assistance with rapid diagnosis and institution of dietary treatment (see Medical Consultants).

### **BLOOD TRANSFUSIONS**

A blood transfusion can seriously interfere with screening tests based on enzymes or other proteins intrinsic to the red blood cell, such as galactose-1-PO<sub>4</sub> uridyl transferase (the deficient enzyme in classical galactosemia), biotinidase, and hemoglobins A, C, F and S. Screening tests for these diseases are valid as early as birth; hence, the screening panel obtained prior to the transfusion will reliably screen for biotinidase deficiency, galactosemia, and hemoglobinopathies, including sickle-cell anemia.

If a newborn requires a blood transfusion even if prior to 24 hours of life, a specimen shall be collected before the blood transfusion. A repeat screening specimen will be required if the initial specimen is collected at < 24 hours of age.

### **PREMATURE, LOW BIRTH WEIGHT AND SICK INFANTS ADMITTED TO THE NICU**

In 2011 the Nebraska Newborn Screening Program adopted via regulation, the Clinical and Laboratory Standards Institute's guidelines for screening of premature, low birth-weight and sick newborns admitted to neonatal intensive care units (I/LA-31-A).

These require:

If a baby is to be transferred to another health care facility, collection of a newborn screen is required prior to transfer. (This has been in regulation since 1996). The NICU is required to verify that the NBS was collected or must draw an admission screen prior to providing any treatments for all premature, low birthweight and sick infants. The "admission screen" is defined as one that must be done before any treatments (with the exception of respiratory) and is targeted towards newborns who are premature, low birthweight or sick. Babies admitted for short term observation who are not going to receive additional treatments are not the intended target of this requirement.

For babies whose first specimen is collected at < 24 hours of age, a repeat shall be collected at 48-72 hours of age.

For babies who are < 2000 grams at birth and whose first specimen was collected at < 24 hours of age, a third specimen should be collected at 28 days of life, or upon discharge if that occurs first.

#### **FAQ's related to NICU admissions:**

1) Does this mean that if a baby's first newborn screen (NBS) happens after 24 hours of age it is not necessary to get a discharge NBS even if they are in the NICU?

Yes if the baby is > 2000 grams at birth. Babies < 2000 grams at birth still need the 28 day or discharge screen. It is probably less likely that the < 2000 gram babies will be getting their screen at > 24 hours of age, but there will be some.

2) What about the baby's with GI obstruction or meconium ileus that might be in the regular nursery for close to 24 hours and then start to show signs of problems. They could easily be transferred to NICU and have their first screen on admission at greater than 24 hours. If this is the case, are they included in the recommendation to get a discharge newborn screen?

No, they are not, if their first screen is normal and was collected at > 24 hours. However, the GI problem still needs to be reported to the newborn screening laboratory at (412) 220-2300 as previously recommended. This is because the IRT used for screening for Cystic Fibrosis may be falsely normal in babies with gastrointestinal obstructions or meconium ileus, and the laboratory will run the 36+ mutation panel as a better screen for CF in these cases.

3) Since the recommendations for serial screening for NICU admissions recommend 3 screens for babies with screens collected at < 24 hours, and < 2000 grams in order to get sufficiently reliable results for the many conditions screened, will the lab report look different?

Yes. All reports for repeat screens collected for babies whose first specimen was collected at < 24 hours will now show results for all conditions screened, not just the amino acid profile and congenital hypothyroidism results. This will include some babies who are discharged at < 24 hours but not transferred to NICU's. This decision was made to standardize and simplify processes in the newborn screening laboratory which helps reduce the opportunity for error.

4) Won't this require more babies to get more specimens than they use to?

The idea behind the standardized practice is to be as certain as we can, that reliable screening results are available for every baby, with the least amount of specimens. In some cases this will prove to reduce the number of specimens required because it will avoid babies getting their first specimen collected post transfusion. In those cases, repeat testing and follow-up gets drawn out several months until the babies own hemoglobin type rather than the transfused hemoglobin is represented in the results.

In other cases, it may be that the third specimen would not otherwise have been collected. The

laboratory and Newborn Screening Program are looking at ways to monitor to see if this becomes a problem. Frequently however, we see babies with drawn early specimens repeated at a few days who then have abnormal screens due to hyperalimentation which is apparent with multiple amino acid elevations on screen results. These babies will require a third screen. The metabolic specialists do not recommend waiting until day 28. In these cases a repeat should be collected 2 days after discontinuing hyperalimentation, or by 14 days regardless of feeding status. When results are significantly abnormal, a confirmatory specimen will usually be recommended.

5) We have a NICU to which we transfer babies in-house. It has been our practice to collect the first newborn screening specimen at > 24 hours to avoid having to repeat. Sometimes the in-house transfers include babies who are < 2000 grams. Does this mean we will now have to collect specimens early, and then collect the repeats?

The “admission screen” is really targeting those babies who are likely to get treatments that can interfere with newborn screening results. If the baby is admitted for close observation but gets no transfusion or hyperalimentation they may be good candidates to wait till > 24 hours to collect their dried blood spot specimen.

The Newborn Screening Program can not approve, endorse or otherwise educate about any practice outside that which is required in the regulations. However, the Program and laboratory will be monitoring the data to see if this results in substantially more babies having early specimens. It may be difficult to distinguish which < 24 hour samples occur because of early discharges or because of in-house NICU transfers. It may be helpful to record on the filter paper “in-house NICU transfer” if possible.

6) Re: “Each hospital NICU should assign a contact person through which newborn screening information can be coordinated”. Should this coordinator be from the lab or NICU? How have other facilities addressed this? We can understand how a coordinator might be helpful in the event of a NICU baby who is still in the facility and needing a re-collection. Would there also be scenarios where the coordinator would play a role for infants that have been discharged?

In Nebraska there are a few hospital NICU’s that have had a coordinator/key contact person for newborn screening issues for years. (That’s where we “borrowed” the idea from!). In one situation the Unit Secretary coordinates communication about newborn screening needs and does an outstanding job. A key element that may be important to making this successful is making sure the coordinator, (whether they are a phlebotomist, nurse or unit secretary) has good access to patient and electronic system information. In higher level NICU’s, it may be the nurses who most often collect the specimens (vs. normal newborn nursery where it is almost always phlebotomy personnel). So perhaps it should be a consideration of whomever is most involved, with the need to know. In addition there can be advantages to having the newborn screening coordinator also have a role in discharge planning and documentation. They are then vested in making sure they document to which health care provider the baby is being discharged so they will know in case additional testing needs to be done. If your NICU patients participate in the TIPS tracking program for the NICU grads, that “coordinator” might also be an important link in educating parents about newborn screening and follow-up as well.

7) We have a sub-set of newborns who are admitted to the NICU for evaluation and are classified as “feeders and growers”. They do not receive any treatments such as blood transfusions or TPN. Many times they come directly from Labor and Delivery and were never admitted to the normal newborn nursery. Do we have to get the newborn screen upon admission to the NICU for these babies or can we wait till they are at least 24 hours of age?

These babies generally are not admitted for higher level NICU care, but for special care beyond the normal nursery where they can be under a little more intensive watchful eye. The NICU may have the latitude within Nebraska’s regulation to wait and collect the specimen after 24 hours. The regulation 2-005.01B states:

at  
any  
"Newborns transferred to neonatal intensive care units (NICU) must have a specimen collected prior to transfer, and information communicated as required at 181 NAC 2-005.01E3. The attending physician the hospital NICU must verify and otherwise ensure a specimen is collected prior to the provision of treatment, excluding respiratory treatment."

If the NICU finds upon admission that the newborn does not already have a specimen collected, the attending physician needs to order the screen and make sure it occurs before treatment. For the sick babies, that's probably immediately. For the well babies generally only admitted for evaluation and as “feeders and growers,” you may be able to wait till 24 hours of age, if tight enough procedures are in place to ensure no treatments occur before the screen is collected.

8) With all these babies getting their newborn screens collected so early now (< 24 hours) we’re seeing a lot more who have abnormal screen results for congenital hypothyroidism (CPH), congenital adrenal hyperplasia (CAH) and cystic fibrosis (CF) on that first screen. Isn’t this more of a burden on the system than before?

This was one of the anticipated results, but since Nebraska has always (since 1996) required specimens to be collected before transfer to the NICU, we did not expect the substantial increase in numbers we are seeing. It is likely that many NICU’s were admitting patients with lower treatment needs (e.g. level 1) and were able to successfully wait until the newborns were at least 24 hours of age before collecting the screen on babies who were not receiving TPN or transfusions. (See information in the above Q&A).

The Nebraska Newborn Screening Program in collaboration with a sub-committee of the Newborn Screening Advisory Committee (Neonatologists and Pediatric Endocrinologists) and with representatives of the screening laboratory changed testing procedures effective 10/3/2011 such that every drawn early specimen will no longer be tested for CAH or CPH. Effective 1/3/12 all drawn early specimens will also no longer be tested for CF. All repeat specimens collected at greater than 24 hours for baby’s whose first specimen was collected at less than 24 hours, will have the entire newborn screening panel tested. and reported.



## **PROGRAM CONTACTS**

**Newborn screening follow-up: 402 471-0374**

**This line is connected to a pager answered after hours and on weekends.**

Krystal Baumert, Follow-up Coordinator (direct line 402 471-0374)

Endocrinopathies, Metabolic Conditions, Drawn early specimens, Home Births, Data

Karen Eveans, Follow-up Specialist (direct line 402 471-6588)

Cystic Fibrosis, Hemoglobinopathies, Post transfusion specimens, unsatisfactory/unacceptable specimens

Julie Luedtke, Program Manager (direct line 402 471-6733)

Newborn Screening systems issues, regulations, patient and professional education, quality assurance, Metabolic formula and food policy and contracts,

Mike Rooney, Administrative Assistant (direct line 402 471-9731)

Supplies of patient education materials, questions on metabolic foods account balances.

Nebraska Newborn Screening Program Web Site: [www.hhss.ne.gov/nsp](http://www.hhss.ne.gov/nsp)

## **Laboratory Contacts**

**(412) 220-2300**

Bill Slimak, Vice President and General Manager

Joseph Quashnock, PhD, Laboratory Director

Bob Wise, Client Services

Dennis Freer, PhD, Consultant, Biochemistry

James DiPerna, PhD, Team Lead, Tandem Mass Spectrometry

Zhili Linn, MD, Molecular

Bethany Sgroi, MS, CGC; Meredith Patik, MS, CGC, Lucy Andrews, MS, CGC, Sara Commander, MS, CGC, Genetic Counselors/results reporting

## **MEDICAL CONSULTANTS**

Medical consultants are available to provide consultation for the follow-up, evaluation, and long-term management of children detected in this program. Questions concerning medical aspects of the program may be directed to the following:

### **Cystic Fibrosis:**

John Colombo, MD, Director,  
Accredited Cystic Fibrosis Center  
University of Nebraska Medical Center CF Clinic  
(402) 559-6275

Heather Thomas, MD  
University of Nebraska Medical Center CF Clinic  
(402) 559-6274

Paul Samut, MD  
University of Nebraska Medical Center CF Clinic  
(402) 559-6274

### **Endocrinology Disorders: Kevin Corley, MD**

University of Nebraska Medical Center  
Pediatric Endocrinology  
Children's Hospital Endocrinology Clinic  
(402) 955-3771

Monina Cabrera, MD  
Children's Hospital Endocrinology Clinic  
(402) 955-3871

Jean-Claude Desmangles, MD  
Children's Hospital Endocrinology Clinic  
(402) 955-3871

### **Hematology Disorders:**

James Harper, MD  
Department of Pediatric Hematology  
University of Nebraska Medical Center  
Children's Hospital Hematology Clinic  
(402) 559-7257

Pediatric Hematology –  
UNMC and Children's Hospital (402) 955-3950

Minnie Abromowitch, MD  
David Gnarra, MD  
Peter F. Coccia, M.D.  
Bruce G. Gordon, M.D.  
Thomas G. Gross M.D., Ph.D.  
James L. Harper, M.D.  
Harold M. Maurer, M.D.  
Phyllis I. Warkentin, M.D.

**Metabolic Disorders:**

William Rizzo, MD  
Munroe-Meyer Institute for Genetics  
Pediatric Metabolism  
University of Nebraska Medical Center  
Children's Hospital Metabolic Clinic  
(402) 559-2560

Richard Lutz, MD  
Munroe-Meyer Institute for Genetics  
Pediatric Metabolism  
University of Nebraska Medical Center  
Children's Hospital Metabolic Clinic  
(402) 559-6800

Eric Rush, MD  
Munroe-Meyer Institute for Genetics  
Pediatric Metabolism  
University of Nebraska Medical Center  
Children's Hospital Metabolic Clinic  
(402) 559-6800

These are the experts of whom we at the NNSP are aware. If this contains any omissions, we apologize and would like to be made aware of anyone who should be included.

## **NEBRASKA TESTING LABORATORY**

### **Where are screening tests done?**

All newborns born in Nebraska have their dried blood spot screening specimens shipped to **PerkinElmer Genetics Inc.** laboratory in Bridgeville, PA. This laboratory was awarded the contract in 2003 and again in 2008 following competitive bid processes. The contract is renewable each year for up to 5 years, to avoid disruption to the Newborn Screening system. The laboratory's phone number is: 412-220-2300.

### **What are the charges for testing?**

The laboratory (during contract years 2008-2013) charges \$38.50 to the hospital for the newborn screen. Hospital charges on top of that are not regulated by contract with the Newborn Screening Program. These charges are generally included in the obstetric DRG billed to insurance or the patient. \$28.50 from each infant screened pays for the laboratory testing for all 28 conditions, filter paper, overnight shipping of specimens to the laboratory, and reporting of results. \$10 of each screen is returned to the Newborn Screening Program and is used to help pay for treatment of metabolic conditions for the metabolic formula and metabolic foods as Statutorily required.

### **What about repeat tests?**

When repeat tests are requested because of a positive or inconclusive result, or because of a drawn early or unsatisfactory/unacceptable specimen, the laboratory does not charge for the repeat testing.

### **What are the requirements for confirmatory testing?**

Confirmatory tests may be done at any laboratory that is CLIA certified and meets the standards for confirmatory test result reporting as specified in Nebraska's regulations. See appendix for regulations 2-007.01E. Many confirmatory tests are sent out to reference laboratories. If the quality of information you receive from confirmatory laboratory test results does not meet these standards, you should contact the NNSP and the submitting hospital or laboratory. Due to factors such as quality of information on laboratory test result reports, quality of data to support neonatal reference ranges, rapidity of turn around time, ability to test submitted confirmatory specimens for DNA on request if confirmatory results are positive, etc. the pediatric subspecialists have experience with high performing laboratories and may identify their preferences for your consideration.

## REFERENCES

- <sup>1</sup> “Newborn Screening Fact Sheets”, Celia I Kaye and the Committee on Genetics, PEDIATRICS 2006; 118;934-963 <http://www.pediatrics.org/cgi/content/full/118/3/e934>
- <sup>2</sup> ACMG Act sheets <http://www.acmg.net>
- <sup>3</sup> Genetic Home Reference <http://ghr.nlm.nih.gov>
- <sup>4</sup> “Toward a Uniform Newborn Screening Panel” Genetics in Medicine, May 2006, Vol 8, Supp 1
- <sup>5</sup> “Newborn Screening of Premature, Low Birth Weight and Sick Newborns, Approved Guideline”, CLSI I/LA 31-A, 2009

Link to NEBRASKA REVISED STATUTES § 71-519 TO 71-524 at [www.dhhs.ne.gov/nsp](http://www.dhhs.ne.gov/nsp)

Link to 181 NAC 2: REGULATIONS GOVERNING SCREENING OF INFANTS FOR METABOLIC DISEASES at [www.dhhs.ne.gov/nsp](http://www.dhhs.ne.gov/nsp)

# Specimen Collection for Newborn Screening

Excerpt from *Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Fifth Edition (LA4-A5)*

## I PREPARATION

- 1.1 Wash hands vigorously.
- 1.2 Wear powder-free gloves and change gloves between infants.
- 1.3 Confirm identity of infant and ensure that all data elements on the form are complete, accurate, and consistent.

## 2 SAMPLING TECHNIQUE

- 2.1 **Warm heel for puncture (incision/stick) site.** Heel warming devices containing an exothermic thermochemical composition are commercially available, or warm site with soft cloth, moistened with warm water (**less than 42 °C**) for three to five minutes. In some situations, warming site may not be necessary to increase blood flow and volume.

- 2.2 Position the infant's leg lower than the heart to increase venous pressure.

- 2.3 Wearing gloves, wipe infant's heel with 70% isopropyl alcohol. \*



- 2.4 Allow heel to air dry.

- 2.5 The puncture should be made within the shaded area as illustrated in the figure to the right. \*



\* Photos reprinted with permission from New York State Department of Health.

† Photo reprinted with permission from GE Healthcare.

- 2.6 Using a sterile lancet of recommended length, perform puncture (depth <2.0 mm) as illustrated or use an incision device. An incision device may provide superior blood flow by making a standardized incision 1.0 mm deep by 2.5 mm long.



- 2.7 Gently wipe off first drop of blood with sterile gauze or cotton ball. (Initial drop contains tissue fluids, which might dilute sample.)

- 2.8 Wait for formation of large blood droplet.

- 2.9 Apply gentle pressure with thumb around the heel but not near the puncture site, and ease intermittently as drops of blood form.

- 2.10 Gently touch the filter paper card to the blood drop and fill each printed circle with a SINGLE application of blood. Apply blood to one side only. Observe the saturation of each printed circle as the blood flows through the filter paper. \*



- 2.11 All used items should be disposed of in an appropriate biohazard container.

- 2.12 After the specimen is collected, elevate the infant's foot and, using sterile gauze or cotton ball, briefly apply gentle pressure to the puncture site until the bleeding stops. Do not apply adhesive bandages.

- 2.13 Allow blood specimen to AIR DRY THOROUGHLY, on a horizontally level, nonabsorbent, open surface, such as a drying rack or plastic-coated test tube rack, for a minimum of three hours at ambient temperature. Keep

specimen away from direct sunlight. (Do not stack or heat.) †



- 2.14 After the specimen has dried, place in an approved container for transport. (See local regulations.)

## 3 PITFALLS

- 3.1 Failure to allow residual alcohol to dry might dilute the specimen and adversely affect test results.
- 3.2 Puncturing the heel on posterior curvature will permit blood to flow away from puncture, making proper spotting difficult. **DO NOT USE PREVIOUS PUNCTURE SITES.**
- 3.3 *Milking* or squeezing the puncture might cause hemolysis and admixture of tissue fluids with specimen.
- 3.4 Do not layer successive drops of blood on the target spot. If blood flow diminishes to incompletely fill circles, **REPEAT** sampling technique 2.1 through 2.10.
- 3.5 Avoid touching the area within the circle before and after blood collection. Do not allow water, feeding formulas, antiseptic solutions, or powder from gloves or other materials to come into contact with the specimen card before or after use.
- 3.6 Do not place the specimens in the transport container until thoroughly dry. Insufficient drying adversely affects test results. Use of sealed plastic bags requires desiccation. Ideally, transport specimens within 24 hours of collection.

Text describing how to collect an acceptable blood spot specimen reprinted with permission from Clinical and Laboratory Standards Institute.

**COLLECTION AND REPORTING (CARE) FORM – NEBRASKA NEWBORN SCREENING PROGRAM**

Note: Filter paper form not to scale.

**ATTACHMENT 3**

**NEBRASKA NEWBORN SCREENING PROGRAM  
NEWBORN TRANSFER FORM**

Date of Transfer: \_\_\_\_\_ Person Completing Form: \_\_\_\_\_

Hospital of Birth: \_\_\_\_\_

Infant's Name: \_\_\_\_\_

Date of Birth: \_\_\_\_\_ Time of Birth: \_\_\_\_\_

Date of Specimen Collection: \_\_\_\_\_ Time of Specimen Collection: \_\_\_\_\_

Transferring Physician: \_\_\_\_\_

Newborn Screening Specimen Collected at Hospital of Birth: Yes No

Newborn Screening Specimen Collected Prior to 24 Hours of Age: Yes No

Infant transfused? Yes No

If yes, was specimen collected prior to transfusion? Yes No

If collected post-transfusion, indicate type: \_\_\_\_\_ and time of transfusion \_\_\_\_:\_\_\_\_

Receiving Hospital: \_\_\_\_\_

Receiving Physician: \_\_\_\_\_

Person Receiving Form: \_\_\_\_\_

**ATTENTION RECEIVING PHYSICIAN: If the newborn screening tests have not been performed or tests need to be repeated when you take charge of the infant, you are responsible for ordering a specimen and returning the results recorded on this form to the hospital of birth.**

Forward one copy of this form to the receiving hospital and fax one copy to:

Nebraska Newborn Screening Program  
Department of Health & Human Services  
P.O. Box 95026  
Lincoln, NE 68509  
Fax 402-471-1863